#### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

November-03-17 10:51 AM

To:

Taylor, Nathan

Subject:

SSHI\_Update Cory AMD\_Nov 3 2017.ppt

Attachments:

SSHI\_Update Cory AMD\_Nov 3 2017.ppt

I am not going to give a formal presentation, but I thought Cory might like to see the information that was provided last to the AMD and SSHI context advisory in June of this year. I will go over some of these findings verbally, and if it is helpful we could look at some specific slides on the teleconference, but I am really thinking this is something he can look at later after hearing about some of this work and findings. Too bad he cannot be here in person...it is much easier to convey that way. It is a bit overwhelming to go over the breadth of what we have done over the phone, and I won't really try. Just the key points—at least that is my intent. I am also interested in answering any questions he might have given conversations with others or what is going on in the media.

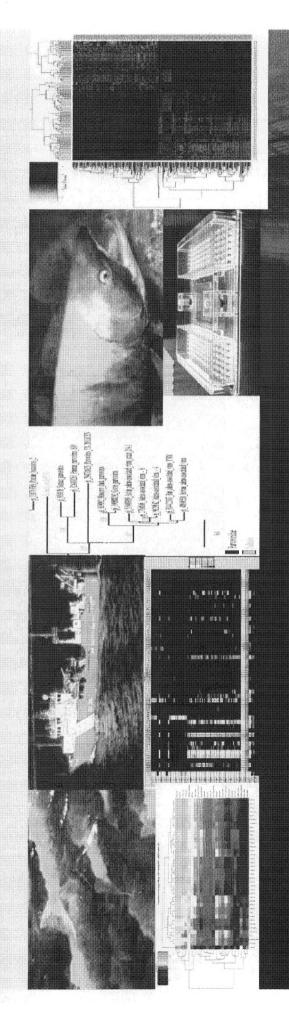
Please forward,

Kristi

# Genome BC Strategic Salmon Health Initiative

Co-led by Brian Riddell (Pacific Salmon Foundation) and Kristi Miller (Fisheries and Oceans Canada) Team includes 16 International Scientists from 6 Universities and 3 governme

7 scientists/postdocs with veterinary degrees (2 pathologists, 5 epidemiologic





Fisheries and Oceans Canada



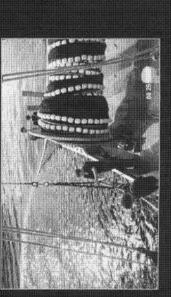


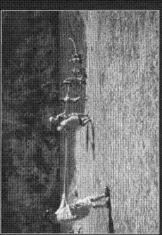


Kristi Miller July 17, 2017

- Infectious agents detected in aquaculture/hatchery/wild salmon in BC
  - Audit samples—merging agents with disease diagnostics
- Development of novel host biomarker panel for viral disease diagnostics (VDD)
- High throughput sequencing detects novel agents
- Salmon Fit Chips
- Next Steps
- Emiliano in situ hybridization/HSMI-PRV research
- Gideon Novel agents







## Objective 1: Field Collections

#### Accomplishments to date

A. Most collections anticipated in support of the program are now completed

Aquaculture - 4,125 (930 Audit, 3,195 SSHI farm)

Wild/Hatchery smolt/juvenile - 39,000 (2008-2016)

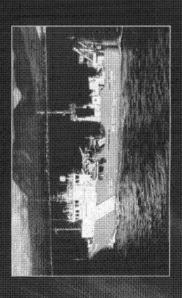
Adults – 8500 sockeye (2005-2016)

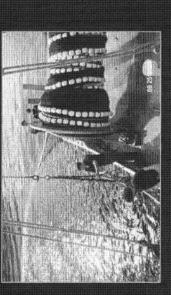
Marine fishes -- ~2000

Hakai has additional smolt samples 2015-16 taken for farm-wild interactions

B. Access Database - Sample inventory and BioMark analysis results

C. Genetic Stock Identification 78% complete (4695/6000 samples)







## Objective 2: Agent Monitoring

### Accomplishments to date

~14,000 samples of 26,000 anticipated run on the BioMark –



Parvicapsula



ethyopthirius



Sphaerothecum



chthyophonus



Ceratomyxa



Parvicapsula

- Data provided to PDFs for analysis and write-up/students writing their
- Three new assays added (Tenacibaculum maritimum, Moritella viscosa, Yersinia ruckeri) and run on Audit, aquaculture and sockeye samples
- Added Viral disease development (VDD) analysis on BioMark (25 dynamic arrays)
- Marine fish currently a focus for analysis



Paranucleospora



Parvicapsula



**Tetracapsuloides** 

Rickettsia

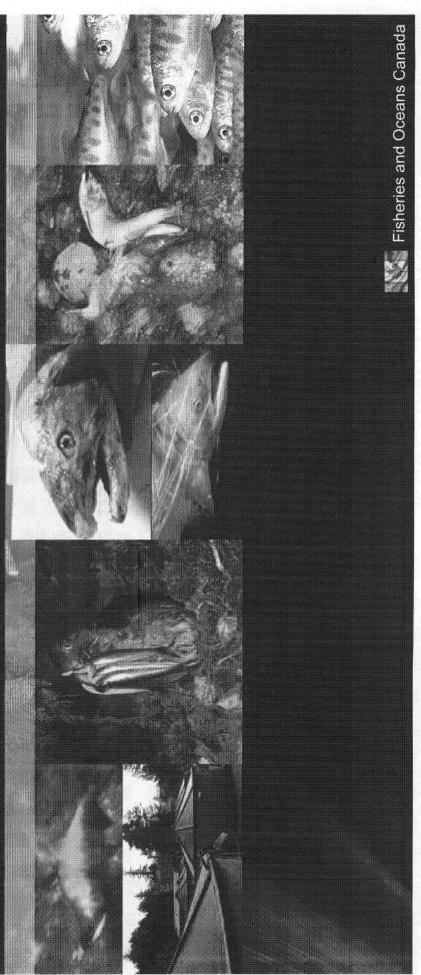


VEN

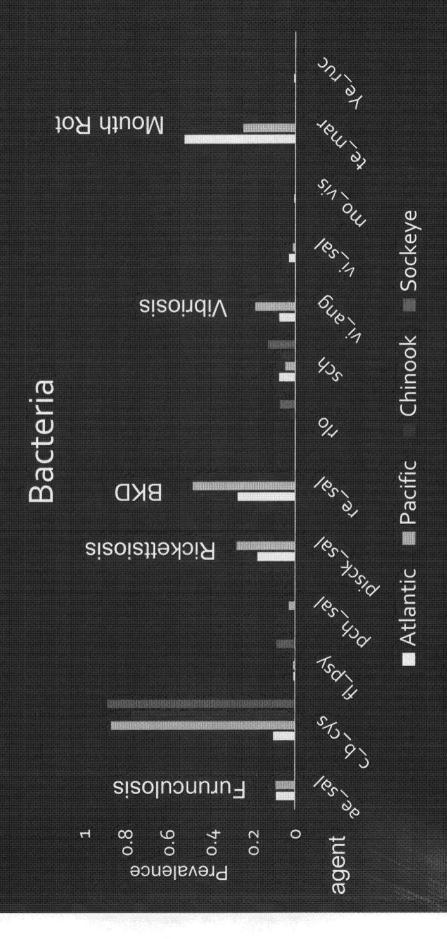


PRV

Infectious Agents Detected in BC Salmon and Herring



Bacterial infections and diseases are generally more common on farms than in migratory fish

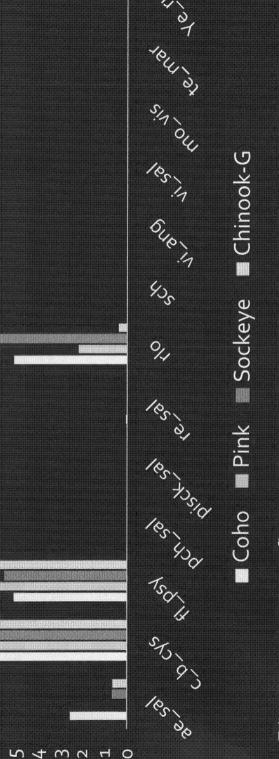


Disease names reflect common diseases on farms caused by the agents detected Certain bacterial infections can become quite prevalent in

returning adult salmon

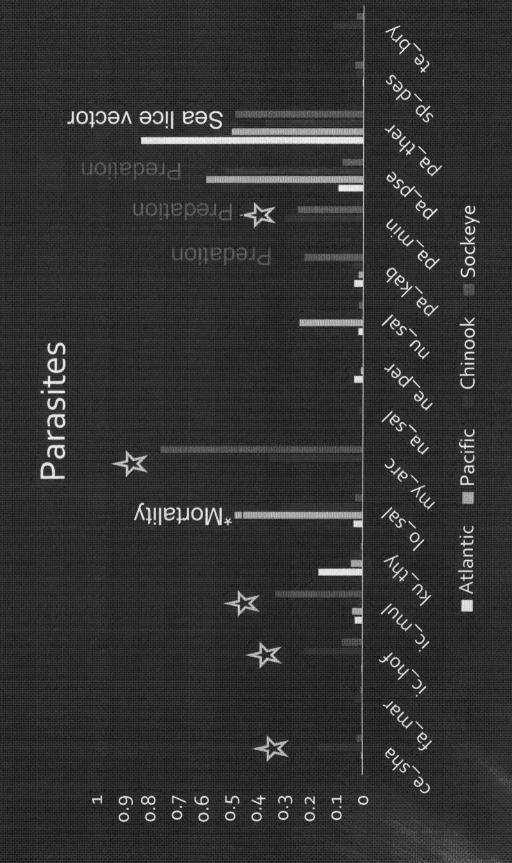
\*Mortality Osmo/stress

Bacteria

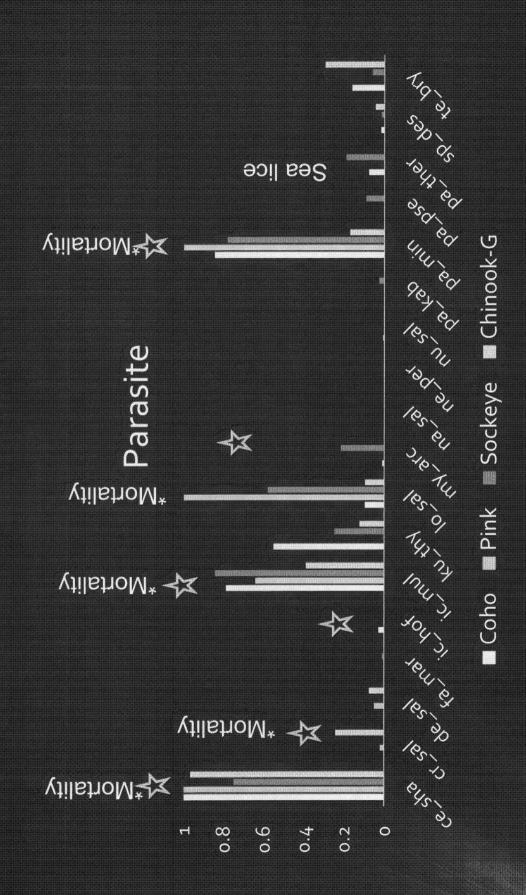


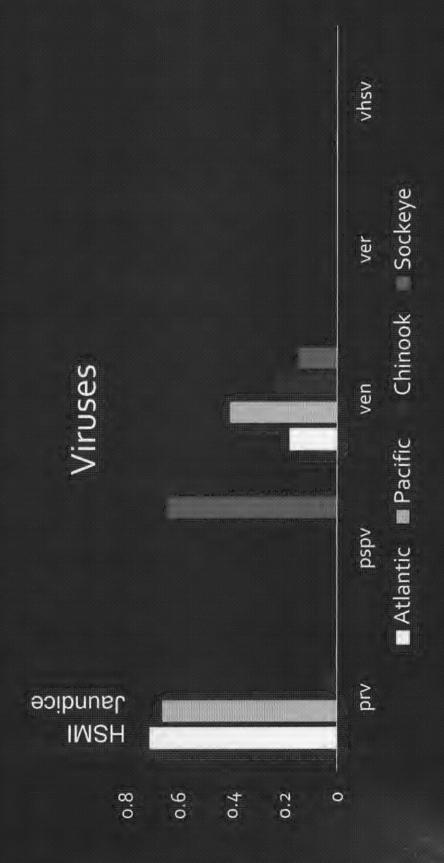
Disease names reflect common diseases on farms caused by the agents detected

common on in migratory fish than on farms and in Pacific versus Atlantic salmon, especially those transmitted in FW (starred) Microparasitic (fungal/protist) infections are generally more



Parasitic infections increase in prevalence in return-migrating adults than in smolts. Agents transmitted in FW are starred





vhsv

0.2 0.3

0.5

9.0

0.1

■ Coho ■ Pink ■ Sockeye ■ Chinook-G

ven

vqeq

prv

# Infectious agents not detected (>1200 samples run)

46 agents initially included in analysis, 3 new agents (known endemics) added later (known impacts on farms) = 49 agents total

40 agents detected in BC salmon. Not detected (OIE reportable)

#### Viruses

ISAv – Infectious Salmon Anemia virus IPNv – Infectious Pancreatic Necrosis virus Omv – Oncorynchus Masu Herpesvirus Sav – Salmon Alphavirus PMCv – Piscine Myocarditis Virus ASPv – Atlantic Salmon Paramyxovirus

#### Bacteria

Aeromonas hydrophila

#### Parasites

Gyrodactylus salaris Myxobolus cerebralis

## PRV highly prevalent in farmed fish (~70% of farm audit samples)

ocean, while the two diseases associated with it occur ~8 months Virus increases in prevalence over the first 6 months in the post ocean-entry

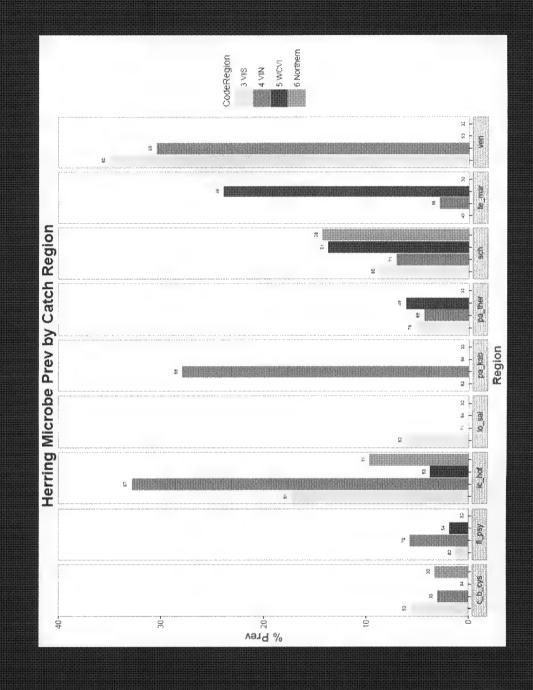


PRV detected, but NOT common, in migratory

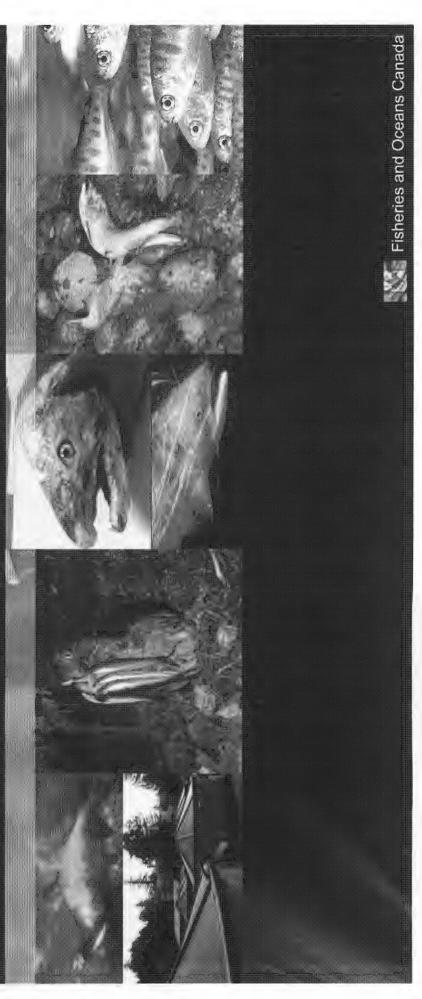
Virus increases from summer through fall/winter in Chinook and Sockeye salmon; 7% overall in Chinook and 3% in Sockeye Mostly in fall/winter



Herring also carry a diversity of agents that infect salmon



Physiological Impact in Migratory Salmon Linking Infectious Agents with Disease Diagnostics in Audit Samples and



### >60% of Audit fish are NOT diagnosed to a specific infectious disease

Some may have died of environmental causes

 Many classified as systemic inflammatory response, no etiological agent

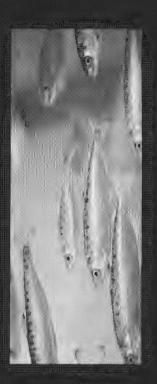




#### Broad-based assessments of infectious agents provides a clearer picture of the relationships between agents and disease

Diagnostics typically employ agent-specific assays when a specific disease is suspected through pathology or clinical signs, so little information on when agents occur in the absence of disease or roles in co-infection





absence of the recognizable lesions associated with the diseases they may cause Audit sample analysis reveals that many Infective agents can be present in the

- 61% of Atlantic salmon and 48% of Chinook salmon with high loads of Renibacterium salmoninarum NOT diagnosed with BKD
- 42% of Atlantic salmon and 69% of Chinook salmon with high loads of Piscirickettsia salmonis NOT diagnosed with Rickettsiosis
- 65% of Atlantic salmon with high loads of Tenacibaculum maritimum NOT diagnosed with Mouth Rot
- 70% of Chinook salmon audits with high PRV loads NOT diagnosed with Jaundice/anemia
- 87% of Atlantic salmon audits with high PRV loads NOT diagnosed with





Audit sample analysis also reveals that in some cases, infective agents associated with diagnosed diseases were not detected: misdiagnosis, undetected strain-variation, recovery?

- 36% of Atlantic salmon and 2% of Chinook salmon diagnosed with BKD had no *Renibacterium salmoninarum* detected
- 23% of Atlantic salmon and 17% of Chinook salmon diagnosed with Rickettsiosis had no *Piscirickettsia salmonis* detected
- 13% of Atlantic salmon diagnosed with Mouth Rot had no Tenacibaculum maritimum detected
- 0% of Chinook salmon audits diagnosed with Jaundice/anemia had no PRV detected
- 3% of Atlantic salmon diagnosed with HSMI (viral cardiomyopathy) had no PRV detected



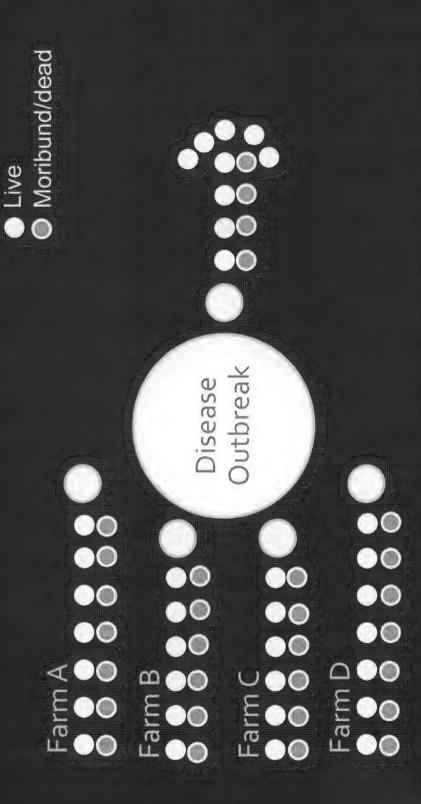


## Longitudinal Farm Study Identifies Emerging Salmon Disease



Clinical pathology Cellular pathology Molecular profiling Pathogen monitoring

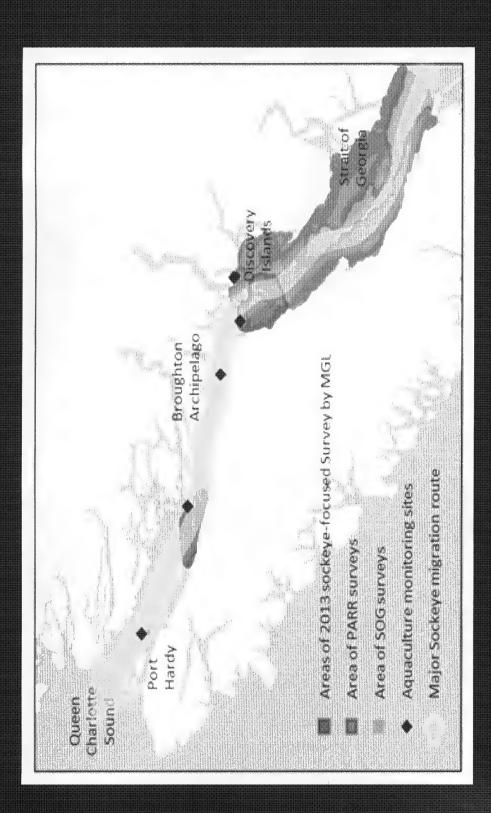
# SSHI Longitudinal Farm Sampling



Fine-scale temporal sampling to uncover cellular and molecular processes associated with to disease development and recovery

Collection of live fish provides best comparator to samples of migratory salmon

# Aquaculture-Wild Interactions



Sampled four geographically dispersed farms over the entire ocean production cycle along the migration pathway of wild salmon emanating from the Fraser River

# Histopathological Investigations - Farm A

Normal Heart

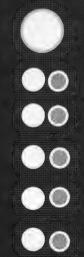
Mild Lesions

Severe

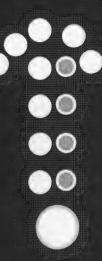
Normal Skele⁴

.amed Skeletal Muscle

# Longitudinal study resolves full developmental pathway of HSMI

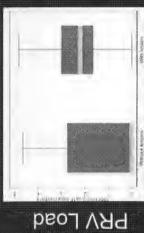


Disease Outbreak



High throughput pathogen monitoring: Identify the shifting pathogen distributions in the heart

P. ther Kudoa



Epidemiological Analyses:

Identify PRV Pathogen Ioads correlated with lesion scores

HSMI

No lesions



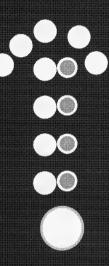
Immunohistochemistry:

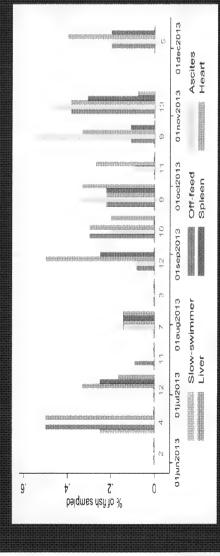
Localization of PRV pathogen within the area of tissue damage

# Longitudinal study resolves full developmental pathway of HSMI

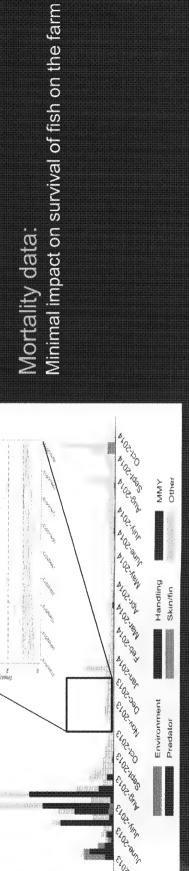


Disease Outbreak



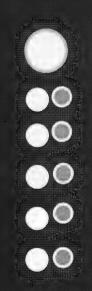


Clinical data: Consistent with HSMI outbreaks in Norway

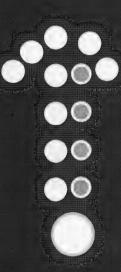


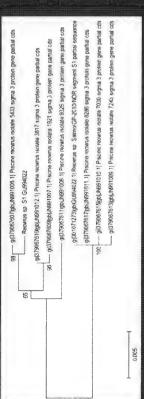
Weekly Mortality (%)

# Longitudinal study resolves full developmental pathway of HSMI



Disease Outbreak





High throughput sequencing:

Full viral genome sequencing identifies PRV strain 99.9% similar to sequences previously observed in wild-migrating BC salmon

Identifying viral transcriptome shifts over disease cycle

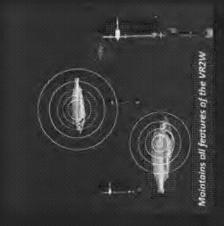


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Transcriptomics (RNA-seq) (Underway): Does the transcriptional profile match HSMI in Norway?

### Which infectious agents carried by migratory salmon are actually impacting them?

Establishing Linkages with Survival



Tracking Studies



Holding Studies



Predation Studies

But... linking pathogens with disease in migratory fish is more difficult

### Which infectious agents carried by migratory salmon are actually impacting them?

### Physiological Impacts

Protein (blood)

Molecular

Cellular

Chinook Smolt Study: Focus on agents showing strong shifts in prevalence/load in the early marine environment

Agents showing strongest physiological impact thus far: P. minibicornis, C. shasta, PRV, Loma

### Novel Viral Disease Diagnostic (VDD) Tool based on host transcriptome



# Molecular Disease Diagnostics – human medicine

#### 

Poscuro.

Conserved Transcriptional Signatures across Integrated, Multi-cohort Analysis Identifies **Multiple Respiratory Viruses** 

Graphical Abstract



Innuthy E. Sweeney, Costina M. Tato. /annick Pouliot, Erika Bongen, west Khatn

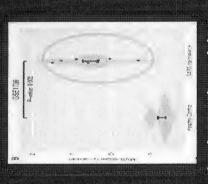
Correspondence

nutable respiratory viruses (MVS) or Sknic ally relevant respiratory viral

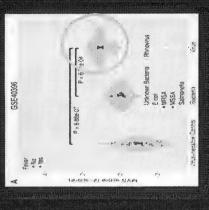
specific to influenza (MAS) by leveraging encogenaty present in public datasets Both signatures distinguish viral from bacterial infections and IMS also

Andres-Terre et al., 2015, Immunity 43, 1199-1211 http://dx.doi.arg/10.1016/j.immunii.2015.11.003 December 15, 2015 \$2015 Elsevier Inc.

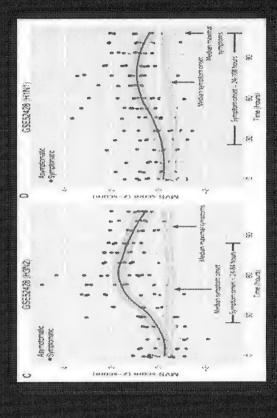
- Mined public transcriptome studies
- Identified diagnostic biomarker signatures for
  - specific to influenza virus respiratory viral disease
    - disease



viral vs. healthy



viral vs. bacterial



Pre-symptomatic viral disease development

Viral Disease Development [VDD]: Salmon RNA viruses

Discovery Analysis—published microarray studies

Krasnov 2011

Skiseol 2011

LeBlanc

2010

ISAV PINCV PRV IPNV Atlantic

Atlantic ANA

**W**S

Atlantic

Published signatures Union (532 features)



**GUNTHER ANALYTICS** Data Analysis, Modeling and Simulation

**Exploration Analysis** 

Gene Shaving, Sparse Independent PCA

Purce 2011

Miller 2007

È

Sockeye Chum Atlantic

Rainbow Trout

2 Z E

VDD 44 biomarker panel

8

VDD Panel can differentiate IHN diseased Sockeye, Chum, and Atlantic salmon in Experimental Challenge Study Atlantic Sockeye

A to 15 samples (injected-hoadKidney) and 35 %. Kidney—primary infective tissue

mortality

mortality

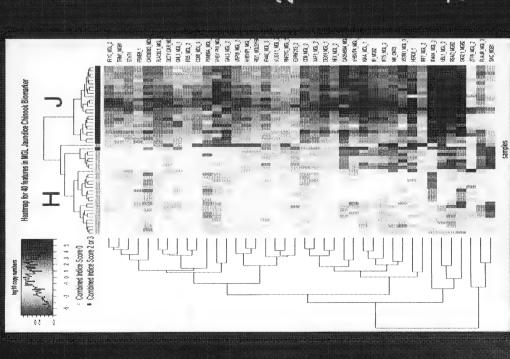
mortality

tissues well before ensuing mortality IHN identified across species and VDD Transcriptional response to

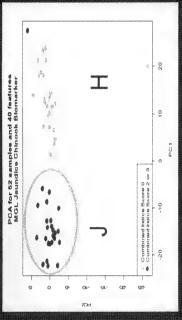
Days Post Challenge

mortality

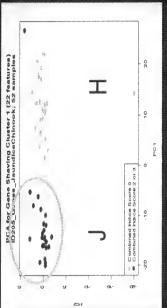
## VDD Panel can differentiate Jaundice from healthy Chinook salmon A viral disease not used in VDD discovery



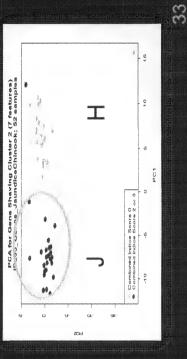
40 biomarkers



22 biomarkers



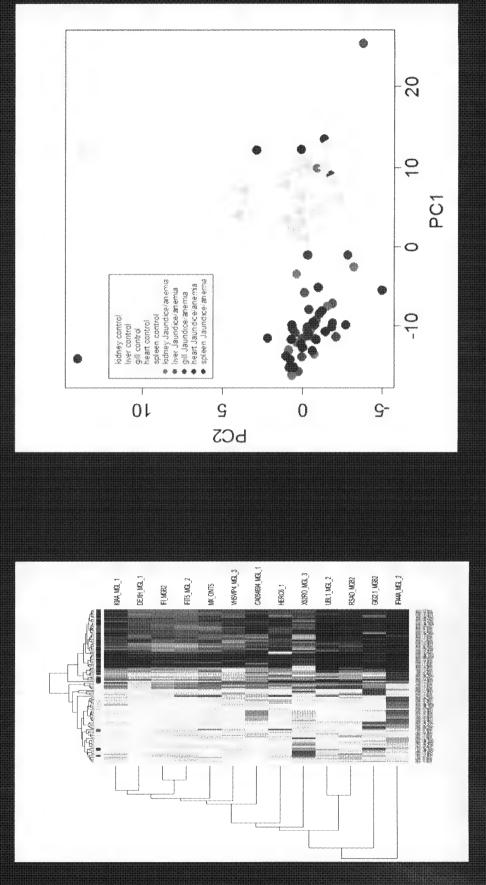
7 biomarkers



Heatmap of all 40 VDD

Differentiates Jaundice from healthy fish Based on expression in Liver

## VDD Panel can differentiate Jaundice from healthy Chinook salmon equally across primary and secondary tissues

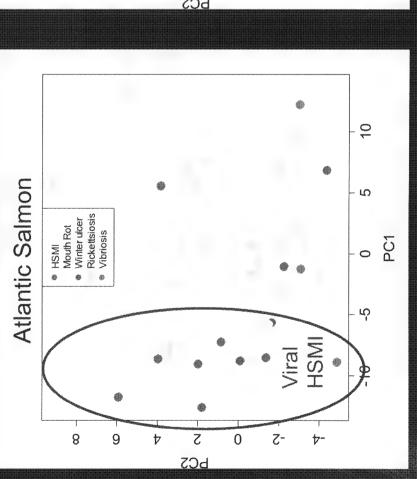


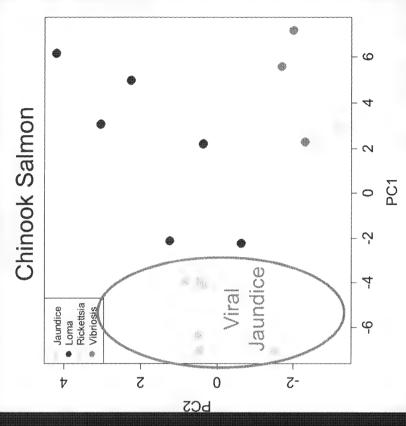
#### 000036

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# Molecular biomarkers – early disease detection

Molecular biomarkers identify differentiate fish with viral versus bacterial diseases

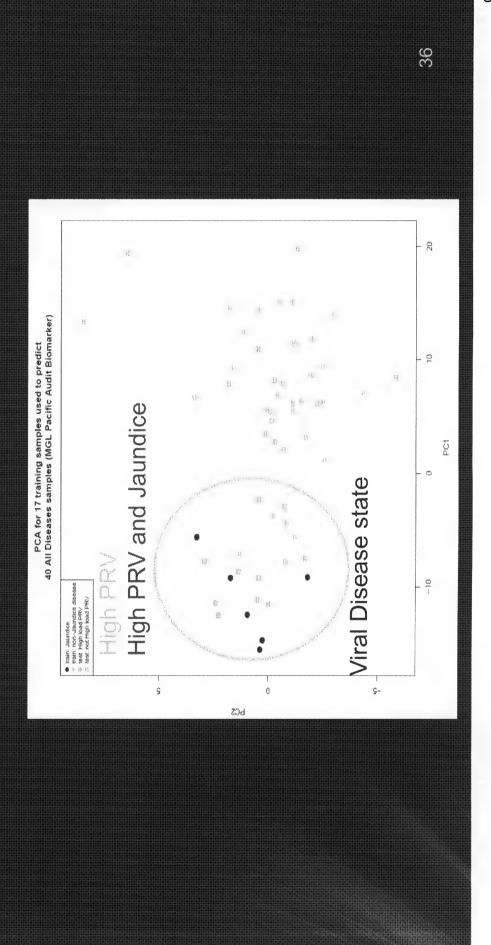




Mixed Tissues, Dead sampled fish, Diagnosed through Veterinary Pathology

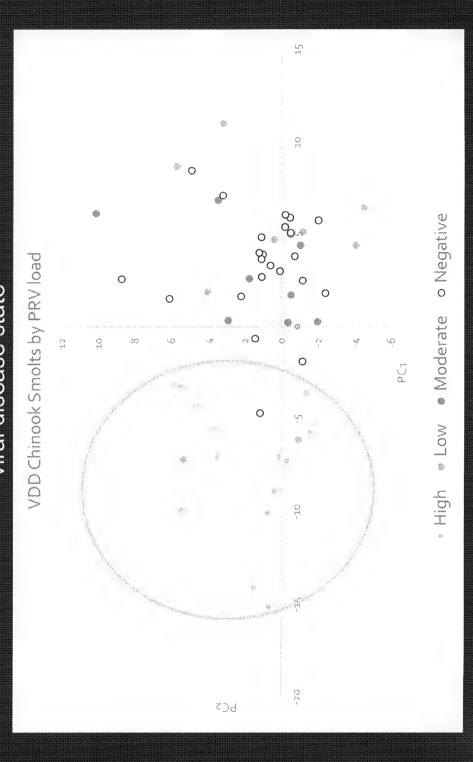
# Molecular biomarkers – Piscine Orthoreovirus Farmed Chinook salmon

80% of farmed Chinook salmon with high loads of PRV are in a "viral disease state"



# Molecular biomarkers – Piscine Orthoreovirus Wild Chinook Salmon

93% of Wild Chinook juveniles containing high loads of PRV are in a 'viral disease state"



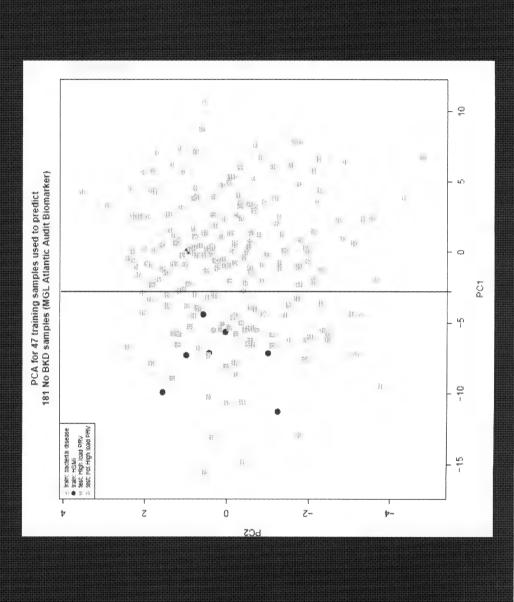
Based on mixed tissue sample

## 00000

# Molecular biomarkers – Piscine Orthoreovirus Farmed Atlantic salmon

50% of Atlantic salmon containing high loads of PRV are in a "viral disease state"

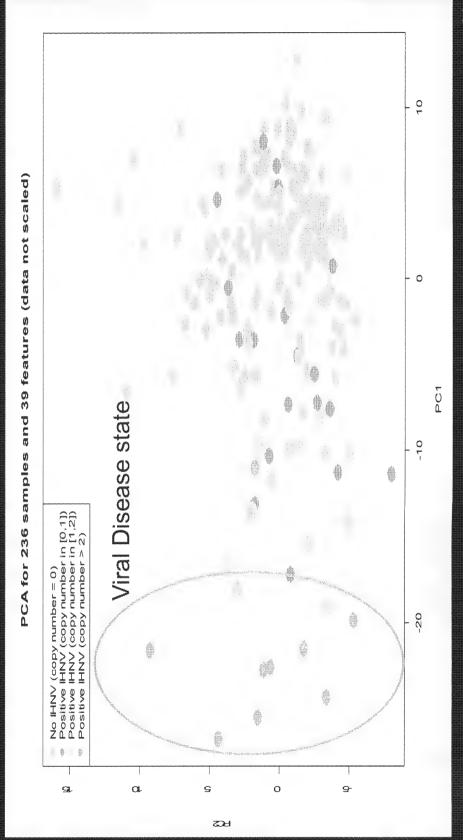
34% of dying Atlantic salmon are in a VDD state—half with unknown viral associations



## 0,000

# Wild Chilko Sockeye Smolts with high loads of IHNv in a viral disease state

89% of Wild Chinook juveniles containing high loads of IHNv are in a "viral disease state"



Non-destructive gill biopsies — ideal for application with tracking studies

g,

## 000041

# Objective 3: Sequencing and Phylogenetics

## Accomplishments to date:

36 High Throughput Sequencing runs

- Agent validation and discovery
  - Agent discovery
- PRV/host Transcriptome









Reovirus

Hepatitis E

Calicivirus

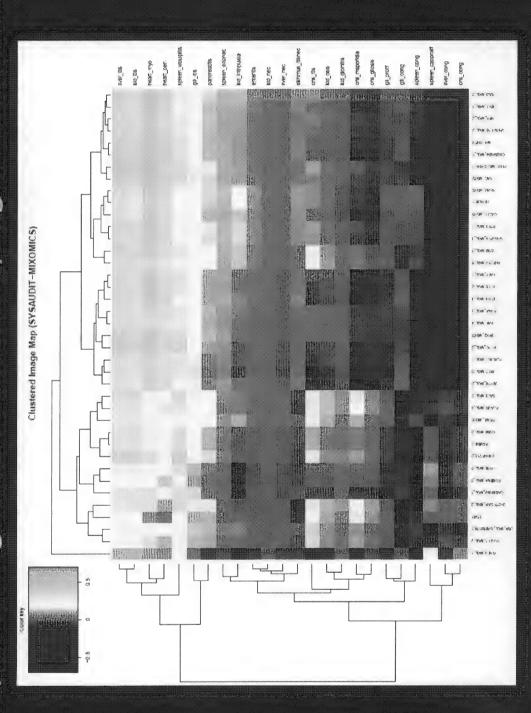
Arenavirus

Coronavirus

Discovery of Novel Salmon Viruses via Targeted High Throughput Sequencing



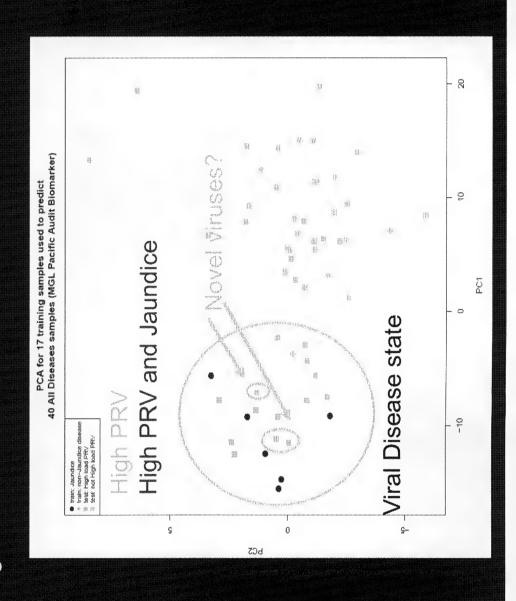
# histopathological lesions in dying farmed salmon VDD panel biomarkers highly correlated with



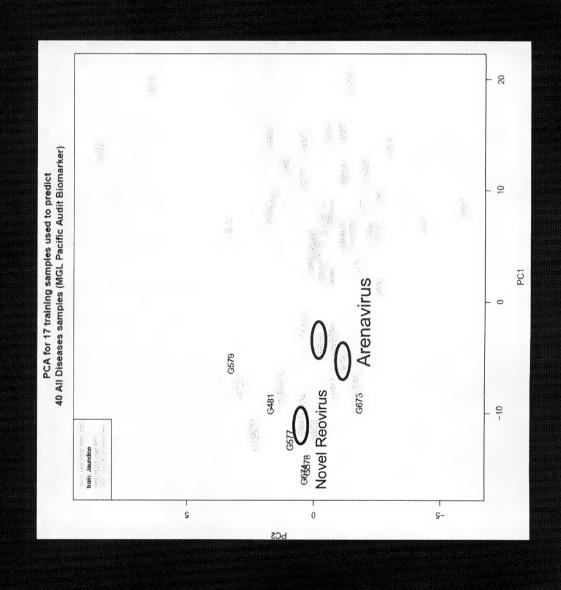
## 000044

# Molecular biomarkers – Piscine Orthoreovirus Farmed Chinook salmon

VDD Panel can be used to identify fish with uncharacterized viruses associated with a developing disease state



## Detection of two novel viruses in farmed Chinook Salmon

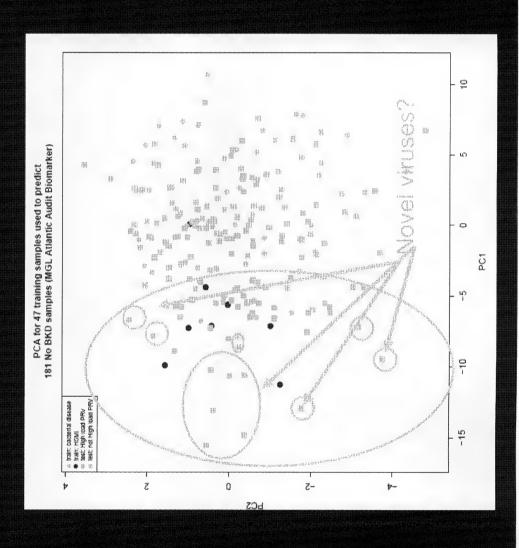


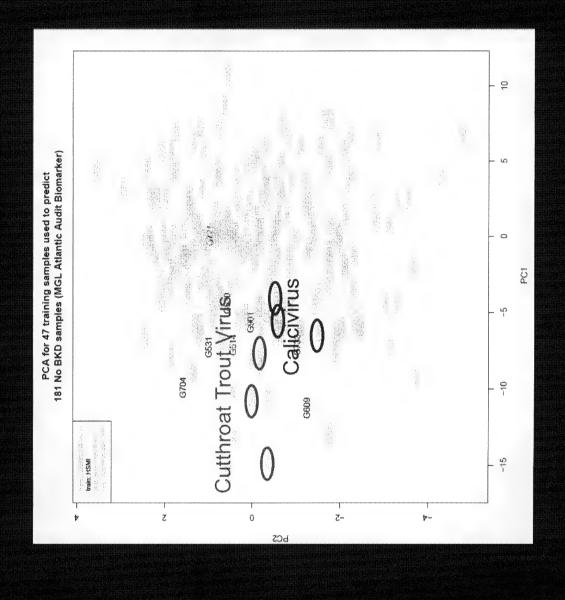
## 000046

# Molecular biomarkers – Piscine Orthoreovirus Farmed Atlantic salmon

50% of Atlantic salmon containing high loads of PRV are in a "viral disease state"

34% of dying Atlantic salmon are in a VDD state—half with unknown viral associations





# Filling in the Audit data with Four Novel Viruses

- Salmon in a VDD state carry significantly more and higher loads of viruses (p<10<sup>6</sup>)
- Average viral load of >10,000 copies/ul compared to <100</li>
- Identified an RNA virus at high load in 85% of fish in a VDD state
- not been diagnosed with a specific disease (many lesions of unknown >1/3<sup>rd</sup> of fish are in a VDD state, and up till now, most of which have etiology)

Overall High Load	73% Atlantic/Pacific	48% 60% Atlantic	71% Atlantic	5% 33% Pacific*	6% 25% Pacific
Prevalence	PRV	Calicivirus	Cutthroat Trout Virus	Arenavirus	Novel Reovirus

# Preliminary analysis of Lesions in fish with Novel Virus detections (N=146 Audits)

- Arenavirus 23% in Pacific salmon; 4% Atlantic
- High loads in Chinook only
- Petechial hemorrhaging on gills, anaemia, brain hemorrhaging
- Novel Reovirus
- 26% in Pacific salmon; 4% Atlantic
- High loads in Chinook only
- Dark spleen, blood filled kidney, anaemia





## Atlantic Salmon Calicivirus

- 17% Pacific salmon; 60% Atlantic
- High loads in Atlantic Salmon only
- Systemic inflammatory disease and visceral petechia, some with resemblance to netpen liver disease, brain hemorrhage, branchitis, ascites



- novel strain 75% similar to known
- 17% Pacific salmon; 92% Atlantic salmon
- High loads only in Atlantic Salmon only; most with strong VDD
- Pathologist noted for some that pathology resembled IHN



# Chinook smolts migrating in SW (N=343)

- Arenavirus
- 10.5% prevalence, 14% of which carry high viral loads
- Highest prevalence Vancouver Island and Thompson River stocks summer/fall
- Targeted for physiological assessments
- Atlantic Salmon Calicivirus
- 1.5% prevalence; all high loads
- Primarily observed in winter/Marble River
- Targeted for physiological assessments
- Cutthroat Trout Virus (novel strain)
- Single low load detection
  - Novel Reovirus
- Single high load detection
- Marble River

Next Steps



# Manuscripts in Development

- Audit data: epidemiological descriptions of agents and pathological lesions comparing distributions in Pacific and Atlantic salmon within and among regions (UPEI)
- Network analysis of Audit data (Gunther Analytics)
- Sockeye salmon infectious agent epidemiology (UPEI)
- Infectious agent distributions between hatchery and wild fish (UPEI)
- Novel viruses (UBC)
- Arenavirus
- VDD application identifies novel viruses in salmon
- Then and now: agents detected 30 years ago compared to present day in returnmigrating sockeye salmon (UPEI
- Assessing molecular, protein and cellular-level physiological associations with infectious agent profiles (UBC)
- Infectious agent responses to high water temperature stress: Holding studies in Fraser River Sockeye salmon (UBC)
- Selective predation on IHNv infected sockeye salmon smolts (UBC)

## Next step research

- RNA-seq study of HSMI development
- Identification of viral proteins stimulated in association with disease development
- Host transcriptome: similarities with studies in Norway, independent assessment of VDD activity across a viral disease outbreak
- Establishing linkages between novel viruses and disease
- Audit sample histopathology, gross lesions, clinical signs, in situ hybridization
- Chinook salmon multi-level physiology
- Re-examination of tracking multiple tracking and holding studies
- Complete analyses of farm samples infectious agents (1600 to go), nistopathology, epidemiological analyses
- Potential to assess farm-level outbreaks of diseases other than HSMI
- Apply to contrast agent distributions between farmed and migratory salmon (principally sockeye)
- Hatchery-wild assessments of agents
- Quinsam
- West Coast of Vancouver Island (Nitinat hatchery)
- Freshwater natal across sockeye, chinook and coho salmon

## Next step research

- In Situ Hybridization studies
- Localizing poorly characterized agents to regions of tissue damage
- **Exploring co-infections**
- Further exploring PRV-HSMI and PRV-Jaundice disease associations
- eDNA: Detection of infectious agents in the water column
- Collaboration with Norway
- Preliminary study underway merging infectious agent and marine fish detections
- Moving towards challenge studies
- Help establish challenge facility at VIU
- Physiological performance end point rather than primary focus on mortality
- Attempt to culture novel viruses
- PRV and novel virus currently of highest interest
- Strong focus of Phase 3 research

### Miller-Saunders, Kristi

From:

Taylor, Nathan

Sent:

November-08-17 7:55 AM

To:

Miller-Saunders, Kristi

Subject:

RE: Creative salmon

Thnx. Will let you know I hear of anything.

----Original Message----

From: Miller-Saunders, Kristi

Sent: Wednesday, November 08, 2017 7:54 AM

To: Taylor, Nathan

Subject: Creative salmon

Just an fyi Kristi

s.21(1)(a)

s.21(1)(b)

### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

November-08-17 9:25 AM

To:

Brian Riddell

Subject:

FW: review of manuscript

Attachments:

Jaundice study manuscript Supplemental Tables and Figure\_V2.docx; Jaundice study

Manuscript\_Sept 2017 Revision\_clean\_V2.docx

FYI

From: - Creative Salmon

Sent: November-08-17 8:11 AM

To: Miller-Saunders, Kristi; Taylor, Nathan; Marty, Gary D AGRI:EX;

**Cc:** - Creative Salmon **Subject:** RE: review of manuscript

Hi Dr. Miller-Saunders,

I can assure you that there is no intent at delay now (nor was there when we were working on this last time).

Multiple calls to discuss a 3 year old manuscript is not to be unexpected.

The degree of financial hardship for the industry or lack there of is not a factor in any of this. I might remind you that Creative Salmon initiated the request for the study in the first place to try to understand why we were seeing a small amount of jaundiced fish.

I can't speak to Gary's or

views on PRV or HSMI.

The manuscript has sat for 3 years, and was shelved by you, and I really appreciate you dusting it off and working on it again. I truly understand the frustration of the constant back and forth on this, then and now, and truthfully would like nothing better then to have this finished, but the reason for the back and forth last time hasn't disappeared because you resurrected the manuscript. There is still some fundamental disagreement on the discussion between coauthors. This has been further compounded by the use of the data from this manuscript and your discussion, in the 2017 paper. (without any of the co-authors knowledge or permission).

Creative Salmon is really in the middle of this, and reliant on scientists and vets for interpretation, and I'm really not sure what we do if the main co-authors disagree. (ie we are not some type of tie breaker) This has never happened to us before in the 25+ years of research we have participated in.

I really think we need some 3<sup>rd</sup> party scientific mediation (does this exist?) that can bring everyone together, and / or have the two interpretations represented in the manuscript.

I will discuss with the other co-authors about getting you the review we've done to date to continue this moving forward, but I really think its going to bring further frustration, and there is not agreement on publishing the manuscript until we can somehow resolve the difference in opinions.

Hidiks,	
Creative Salmon Co. Ltd. T 250-725-2884 F 250-725-2885 creativesalmon.com	
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From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]	NY SINSTENSES NOONANA AMIN'NY FIVONDRANDRA NA PARANTANA MANDERANDRA MARIA AMIN'NY TAONA MANDRA MARIA AMIN'NY M
<b>Sent:</b> Tuesday, November 07, 2017 7:32 PM <b>To:</b> Creative Salmon; Taylor, Nathan	
Cc: - Creative Salmon	
Subject: RE: review of manuscript	
This does not work for me and is appearing to be very similar to past patterns of d Gary or so I am not clear on their intent.	elay. I have heard nothing from either
I am really not sure what should take multiple calls when the only substantive chainclusion of recent publications that added context to our findings. We spent almo previously.	
HSMI and jaundice are not causing such financial hardship to the industry that you resolve, but our research beyond this study is suggestive of a consistent role of PR diseases. I have not overly played this role in the manuscript, although I do prese PRV to jaundice in Pacific salmon in Norway, Chile, and Japan.	V in the development of both of these
I too have a very busy schedule and will be away much of December, and I have e before I go away.	very intent to get this paper submitted
Kristi	
From: - Creative Salmon	mii slame v. uu van darballed allar thaldist thillids thidas i thillidi thillid jihilid ja propagat
<b>Sent:</b> November 6, 2017 7:46 AM	s.19(1)
To: Miller-Saunders, Kristi; Taylor, Nathan  Cc: - Creative Salmon	s.21(1)(b)
Subject: RE: review of manuscript	

Open to suggestions on a path forward.

Hi Dr. Miller-Saunders,

I'm sorry, we unfortunately will not have the review of the manuscript completed by this week.

As mentioned, it's a very busy time for everyone. Our next call to discuss this will be in the week of Nov 20<sup>th</sup>, and hope to get back to you shortly after that.

Best Regards,

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From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

Sent: Monday, October 30, 2017 2:29 PM

**To:** Creative Salmon; Taylor, Nathan

**Cc:** - Creative Salmon **Subject:** FW: review of manuscript

I accidentally did not press reply all.

Kristi

From: Miller-Saunders, Kristi
Sent: October-30-17 2:29 PM
To: - Creative Salmon'
Subject: RE: review of manuscript

Thank you for getting back to me At this point, I would not expect extensive edits to the intro, methods, or results, as we have hashed these out extensively in the past and nothing substantive has changed, and I have incorporated almost all of the suggestions from previous comments. In answer to your question, yes, we re-analysed all of your samples with our full agent panel, after the validation of the platform, and using the same methods we have applied on over 16,000 samples to date.

I would appreciate your getting your comments back by next Wed.

Thanks,

s.19(1)

Kristi

From: - Creative Salmon [

Sent: October-30-17 2:14 PM

To: Miller-Saunders, Kristi; - Creative Salmon

Cc: Taylor, Nathan

Subject: review of manuscript

Hi Dr. Miller,

We just wanted to touch base to let you know that we have been working on our review of the jaundice study manuscript and had hoped to get you comments/edits by the end of this month but unfortunately we will need a few additional days. We have had a couple of calls already and have another scheduled for Friday morning to review and discuss our edits and can hopefully get you the reviewed draft early next week.

We do have a question, were the samples from the study re-analyzed with your system since once it had been validated?

Regards,

Creative Salmon Co. Ltd.

F 250-725-2885

creativesalmon.com



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s.19(1)

Supplemental Table 1. TaqMan assay references for infectious agents assessed on the Fluidigm BioMark<sup>TM</sup> HD platform. See Miller et al. 2015 for full references and TaqMan assay details.

Infectious Agent	Туре	Assay Abbreviation	Assay Reference
Aeromonas hydrophila	Bacterium	ae_hyd	Lee et al. 2006
Aeromonas salmonicida	Bacterium	ae_sal	modified from Keeling et al. 2013
Candidatus Branchiomonas cysticola	Bacterium	c_b_cys	Mitchell et al. 2013
Flavobacterium psychrophilum	Bacterium	fl_psy	Duesund et al. 2010
Gill chlamydia	Bacterium	sch	Duesund et al. 2010
Piscichlamydia salmonis	Bacterium	pch_sal	Nylund et al. 2008
Piscirickettsia salmonis	Bacterium	pisck sal	Corbeil et al. 2003
Renibacterium salmoninarum	Bacterium	re_sal	Powell et al. 2005
Rickettsia-like organism	Bacterium	rlo	Lloyd et al. 2011
Vibrio anguillarum	Bacterium	vi_ang	Miller et al. 2015
Vibrio salmonicida	Bacterium	vi_sal	Miller et al. 2015
Nanophyetus salmincola	Fluke	na sal	Miller et al. 2015
Ceratomyxa shasta	Parasite	ce_sha	Hallett and Bartholomew 2006
Cryptobia salmositica	Parasite	cr_sal	Miller et al. 2015
Dermocystidium salmonis	Parasite	de sal	Miller et al. 2015
- -acilispora margolisi	Parasite	fa_mar	Miller et al. 2015
Gyrodactylus salaris	Parasite	gy_sal	Collins et al. 2010
chthyophonus hoferi	Parasite	ic_hof	White et al. 2013
chthyophthirius multifiliis	Parasite	ic_mul	Miller et al. 2015
Kudoa thyrsites	Parasite	ku thy	Funk et al. 2007
.oma sp.	Parasite	lo_sal	Miller et al. 2015
Myxobolus arcticus	Parasite	my_arc	Miller et al. 2015
Myxobolus cerebralis	Parasite	my_cer	Kelley et al. 2004
Myxobolus insidiosus	Parasite	my_ins	Miller et al. 2015
Neoparamoeba perurans	Parasite	ne_per	Fringuelli <i>et al</i> . 2012
Nucleospora salmonis	Parasite	nu_sal	Foltz et al. 2009
Paranucleospora theridion	Parasite	pa_ther	Nylund <i>et al</i> . 2010
Parvicapsula kabatai	Parasite	pa_trier pa_kab	Miller et al. 2015
Parvicapsula Mabatai Parvicapsula minibicornis	Parasite	pa_kab pa_min	Hallett and Bartholomew 2009
Parvicapsula minibicomis Parvicapsula pseudobranchicola	Parasite	pa pa_pse	
•	Parasite	sp_des	Jørgensen <i>et al</i> . 2011 Miller et al . 2015
Sphaerothecum destructuens Spironucleus salmonicida	Parasite		Miller et al. 2015
•	Parasite	sp_sal	
Tetracapsuloides bryosalmonae	Virus	te_bry	Bettge et al. 2009 Nylund et al. 2008
Atlantic salmon paramyxovirus		aspv	Purcell et al. 2013
nfectious hematopoietic necrosis virus nfectious pancreatic necrosis virus	Virus Virus	ihnv	Clouthier et al. 2014
nfectious paricreatic flectosis virus	Virus	ipnv Snow7	Snow et al. 2006
nfectious saimon anemia virus		isav8	LeBlanc et al. 2010
Pacific salmon parvovirus	Virus Virus		Miller et al. 2015
•		pspv pspv1	
Piscine myocarditis virus (CMS)	Virus	pmcv1	Wiik-Nielsen et al. 2013 Wiik-Nielsen et al. 2012
Piscine reovirus (HSMI)	Virus	prv	
Salmon alphavirus 1, 2, and 3	Virus	sav	Andersen et al. 2007
Salmonid herpesvirus / Oncorhynchus	Virus	omv	Miller et al. 2015
/iral encephalopathy and retinopathy	Virus	ver	Korsnes et al. 2005
Erythrocytic necrosis virus	Virus	env	Purcell et al. 2016
/iral hemorrhagic septicemia virus	Virus	vhsv1 g hkg	Jonstrup et al. 2013

## Supplemental Table 2. Stocking information for farms A and B.

Farm A	Farm B
May, 2004- February 2006	October 2005 - May 2007
May 2008 - January 2010	September 2007 - August 2009
May 2010 - January 2012	September 2009 - October 2011

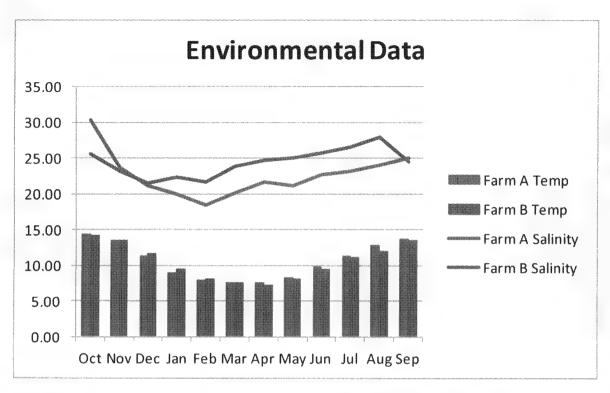
## Pages 63 to / à 66 are withheld pursuant to sections sont retenues en vertu des articles

20(1)(b), 20(1)(c)

of the Access to Information Act de la Loi sur l'accès à l'information

Pen #4 November 2010	Pen #6 November 2010	Pen #8 January 2011
2	2	6
Pen #3 January 2011	Pen #5 December 2010	Pen #7 December 2010
6	4	4
	November 2010  2  Pen #3	November 2010  2  Pen #3  November 2010  Pen #5

Supplemental Figure 2. The farm cage system at Farm A. Pens 1 and 2 were closest to land. The dates and large numerals list the month, year, and order when IJAS was first observed in the pens.



Supplemental Figure 3. Mean water Temperature and Salinity for Farm A and B (May 2010 - Sept 2011) at 6 m.

## Pages 69 to / à 71 are withheld pursuant to sections sont retenues en vertu des articles

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1 Histopathology and genomic characterization of idiopathic 2 jaundice and anemia syndrome in cultured Chinook salmon 3 (Oncorhynchus tshawytscha) 4 5 6 Kristina M. Miller\*1, Karia H. Kaukinen1, Shaorong Li1, Angela Schulze1, Barbara Cannon2, 7 Tim Rundle<sup>2</sup>, Gary D. Marty<sup>3</sup>, Sonja M. Saksida<sup>4</sup> 8 9 10 <sup>1</sup>Molecular Genetics, 11 Fisheries and Oceans Canada 12 **Pacific Biological Station** 13 3190 Hammond Bay Rd 14 Nanaimo, BC V9T 6N7 15 250-756-7155 16 Kristi.miller@dfo-mpo.gc.ca 17 18 <sup>2</sup>Creative Salmon Company Ltd. 19 PO Box 265 20 Tofino, British Columbia 21 22 23 <sup>3</sup>Animal Health Centre, 24 Ministry of Agriculture, 25 Abbotsford, BC, Canada 26 27 British Columbia Centre for Aquatic Health Sciences 28 Campbell River, BC, Canada V9W 5B1 29 Comment [D1]: to revise with current address 30 31 \*corresponding author 32 33 Keywords: jaundice syndrome, IJAS, anemia, salmon, aquaculture, piscine orthoreovirus, 34 microarray, gene expression profiling, anti-viral

1

## Pages 73 to / à 130 are withheld pursuant to sections sont retenues en vertu des articles

20(1)(b), 20(1)(c)

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### Miller-Saunders, Kristi

From: Sent:	Miller-Saunders, Kristi November-08-17 9:19 AM
То:	Taylor, Nathan
Cc:	Thomson, Andrew
Subject:	FW: review of manuscript
FYIManuscript was ser	nt back to industry September 29, 2017.
I need this resolved.	
Kristi	
<b>Sent:</b> November-08-17 <b>To:</b> Miller-Saunders, Kris <b>Cc:</b> - Creat	sti; Taylor, Nathan; Marty, Gary D AGRI:EX; : State of the control
Subject: RE: review of	manuscript
Hi Dr. Miller-Saunders,	
I can assure you that the	ere is no intent at delay now (nor was there when we were working on this last time).
Multiple calls to discuss	a 3 year old manuscript is not to be unexpected.
-	nardship for the industry or lack there of is not a factor in any of this. I might remind you that d the request for the study in the first place to try to understand why we were seeing a small h.
I can't speak to Gary's o	views on PRV or HSMI.
again. I truly understand nothing better then to have you resurrected the manauthors. This has been to	for 3 years, and was shelved by you, and I really appreciate you dusting it off and working on it d the frustration of the constant back and forth on this, then and now, and truthfully would like ave this finished, but the reason for the back and forth last time hasn't disappeared because nuscript. There is still some fundamental disagreement on the discussion between cofurther compounded by the use of the data from this manuscript and your discussion, in the my of the co-authors knowledge or permission).

Creative Salmon is really in the middle of this, and reliant on scientists and vets for interpretation, and I'm really not sure what we do if the main co-authors disagree. (ie we are not some type of tie breaker) This has never happened to us before in the 25+ years of research we have participated in.

I really think we need some 3<sup>rd</sup> party scientific mediation (does this exist?) that can bring everyone together, and / or have the two interpretations represented in the manuscript.

s.19(1)

I will discuss with the other co-authors about getting you the review we've done to date to continue this moving forward, but I really think its going to bring further frustration, and there is not agreement on publishing the manuscript until we can somehow resolve the difference in opinions.

Open to suggestions on a path forward.

Thanks,

Creative Salmon Co. Ltd. T 250-725-2884 F 250-725-2885 creativesalmon.com

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From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.qc.ca]

Sent: Tuesday, November 07, 2017 7:32 PM

Creative Salmon; Taylor, Nathan To:

Cc: - Creative Salmon Subject: RE: review of manuscript

This does not work for me and is appearing to be very similar to past patterns of delay. I have heard nothing from either so I am not clear on their intent. Gary or

I am really not sure what should take multiple calls when the only substantive changes to the manuscript were the inclusion of recent publications that added context to our findings. We spent almost 3 years going through this study previously.

I recognize that perhaps

HSMI and jaundice are not causing such financial hardship to the industry that you deem it important enough to fully resolve, but our research beyond this study is suggestive of a consistent role of PRV in the development of both of these diseases. I have not overly played this role in the manuscript, although I do present briefly the recent studies linking PRV to jaundice in Pacific salmon in Norway, Chile, and Japan.

I too have a very busy schedule and will be away much of December, and I have every intent to get this paper submitted before I go away.

Kristi

From: **Sent:** November 6, 2017 7:46 AM

Creative Salmon

s.19(1)

s.21(1)(b)

Cc: Creative Salmon Subject: RE: review of manuscript Hi Dr. Miller-Saunders, I'm sorry, we unfortunately will not have the review of the manuscript completed by this week.  As mentioned, it's a very busy time for everyone. Our next call to discuss this will be in the week of Nov 20th, and hope to get back to you shortly after that.  Best Regards,  Creative Salmon Co. Ltd. T 250-725-2884 F 250-725-2885 Treativesalmon.com  The contents of this email and any attachments are confidential and may be privileged or otherwise protected from disclosure. If you are not the intended recipient, any reading, use, disclosure, copying or distribution of all or parts of this email or associated attachments is strictly prohibited. If you are not an intended recipient, please notify the sender immediately by replying to this message or by telephone and delete this e-mail and any attachments permanently from your system  From: Miller-Saunders, Kristi [mailto;Kristi,Saunders@dfo-mpo.gc.ca] Sent: Monday, October 30, 2017 2:29 PM To: Creative Salmon; Taylor, Nathan Dec: -Creative Salmon Taylor Tayl		
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Creative Salmon Co. Ltd. T 250-725-2884 F 250-725-2885 Creative Salmon Comments of this email and any attachments are confidential and may be privileged or otherwise protected from disclosure. If you are not the intended recipient, any reading, use, disclosure, copying or distribution of all or parts of this e-mail or associated attachments is strictly prohibited. If you are not an intended recipient, please notify the sender immediately by replying to this nessage or by telephone and delete this e-mail and any attachments permanently from your system  From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca] Sent: Monday, October 30, 2017 2:29 PM To: Creative Salmon; Taylor, Nathan Cc: - Creative Salmon; Taylor, Nathan Cc: - Creative Salmon Taylor, Nathan Cristi  From: Miller-Saunders, Kristi Sent: October-30-17 2:29 PM To: - Creative Salmon'	I'm sorry, we unfortunately will not have the review of the manuscript completed by this week.	
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I would appreciate your getting your comments back by next Wed.

s.19(1)

Thanks,

Kristi

**From:** - Creative Salmon

Sent: October-30-17 2:14 PM

**To:** Miller-Saunders, Kristi; - Creative Salmon

Cc: Taylor, Nathan

Subject: review of manuscript

Hi Dr. Miller,

We just wanted to touch base to let you know that we have been working on our review of the jaundice study manuscript and had hoped to get you comments/edits by the end of this month but unfortunately we will need a few additional days. We have had a couple of calls already and have another scheduled for Friday morning to review and discuss our edits and can hopefully get you the reviewed draft early next week.

We do have a question, were the samples from the study re-analyzed with your system since once it had been validated?

Regards,

Creative Salmon Co. Ltd.

.

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Is a phone call to sort this out possible? I worry about the conflict escalating by email and I have the sense that you managed to smooth things over on the last go around.  From: - Creative Salmon Sent: Wednesday, November 08, 2017 8:11 AM To: Miller-Saunders, Kristi; Taylor, Nathan; Marty, Gary D AGRI:EX; Cc: - Creative Salmon Subject: RE: review of manuscript  Hi Dr. Miller-Saunders, I can assure you that there is no intent at delay now (nor was there when we were working on this last time).  Multiple calls to discuss a 3 year old manuscript is not to be unexpected.  The degree of financial hardship for the industry or lack there of is not a factor in any of this. I might remind you the Creative Salmon initiated the request for the study in the first place to try to understand why we were seeing a sma amount of jaundiced fish.  I can't speak to Gary's or sviews on PRV or HSMI.	From: Sent: To: Subject:	Taylor, Nathan November-08-17 8:14 AM Miller-Saunders, Kristì FW: review of manuscript
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	I can't speak to Gary's	s views on PRV or HSMI.

The manuscript has sat for 3 years, and was shelved by you, and I really appreciate you dusting it off and working on it again. I truly understand the frustration of the constant back and forth on this, then and now, and truthfully would like nothing better then to have this finished, but the reason for the back and forth last time hasn't disappeared because you resurrected the manuscript. There is still some fundamental disagreement on the discussion between coauthors. This has been further compounded by the use of the data from this manuscript and your discussion, in the 2017 paper. (without any of the co-authors knowledge or permission).

Creative Salmon is really in the middle of this, and reliant on scientists and vets for interpretation, and I'm really not sure what we do if the main co-authors disagree. (ie we are not some type of tie breaker) This has never happened to us before in the 25+ years of research we have participated in.

I really think we need some 3<sup>rd</sup> party scientific mediation (does this exist?) that can bring everyone together, and / or have the two interpretations represented in the manuscript.

I will discuss with the other co-authors about getting you the review we've done to date to continue this moving forward, but I really think its going to bring further frustration, and there is not agreement on publishing the manuscript until we can somehow resolve the difference in opinions. s.19(1)

Open to suggestions on a path forward.
Thanks,
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From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]  Sent: Tuesday, November 07, 2017 7:32 PM  To: Creative Salmon; Taylor, Nathan  Cc: - Creative Salmon  Subject: RE: review of manuscript
This does not work for me and is appearing to be very similar to past patterns of delay. I have heard nothing from either Gary or so I am not clear on their intent.
I am really not sure what should take multiple calls when the only substantive changes to the manuscript were the inclusion of recent publications that added context to our findings. We spent almost 3 years going through this study previously.  I recognize that perhaps HSMI and jaundice are not causing such financial hardship to the industry that you deem it important enough to fully resolve, but our research beyond this study is suggestive of a consistent role of PRV in the development of both of these diseases. I have not overly played this role in the manuscript, although I do present briefly the recent studies linking PRV to jaundice in Pacific salmon in Norway, Chile, and Japan.
I too have a very busy schedule and will be away much of December, and I have every intent to get this paper submitted before I go away.
Kristi
From: - Creative Salmon  Sent: November 6, 2017 7:46 AM  To: Miller-Saunders, Kristi; Taylor, Nathan  Cc: - Creative Salmon  Subject: RE: review of manuscript  s.19(1)

s.21(1)(b)

Hi Dr. Miller-Saunders,

I'm sorry, we unfortunately will not have the review of the manuscript completed by this week.

As mentioned, it's a very busy time for everyone. Our next call to discuss this will be in the week of Nov 20<sup>th</sup>, and hope to get back to you shortly after that.

Best Regards,



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From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

Sent: Monday, October 30, 2017 2:29 PM

To: Creative Salmon: Taylor, Nathan

**Cc:** - Creative Salmon **Subject:** FW: review of manuscript

I accidentally did not press reply all.

Kristi

From: Miller-Saunders, Kristi
Sent: October-30-17 2:29 PM
To: Creative Salmon'

**Subject:** RE: review of manuscript

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I would appreciate your getting your comments back by next Wed.

Thanks,

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**To:** Miller-Saunders, Kristi; - Creative Salmon

Cc: Taylor, Nathan

Subject: review of manuscript

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We do have a question, were the samples from the study re-analyzed with your system since once it had been validated?

Regards,

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#### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

November-11-17 10:42 AM

To:

cory.jackson@dfo-mpo.gc.ca

Cc:

Taylor, Nathan

Subject:

Jaundice Manuscript

**Attachments:** 

Jaundice study Manuscript\_Sept 2017 Revision\_clean\_V2.docx

Cory,

I believe I promised to forward you the jaundice paper that we discussed a week ago FYI. If you have any questions, please do not hesitate to contact me. We

are presently conducting analyses that localize PRV within the area of tissue damage in the liver and kidney tissue diagnostic of the disease. We are planning on writing up a short communication of these findings as a separate study within the next month or so, and I will share it at that time.

Have a good weekend,

Kristi

s.21(1)(b)

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are duplicates of
sont des duplicatas des
pages 72 to / à 130

#### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

November-15-17 9:33 AM

To:

Parsons, Jay

Cc:

Taylor, Nathan

Subject:

FYI

Attachments:

Jaundice study Manuscript\_Sept 2017 Revision\_clean\_V2.docx

Hello Jay,

I heard from Nathan that Creative Salmon contacted you about the revision of the Jaundice manuscript I sent back to them in September and that I intend to move forward to publication. I was not sure if they provided you a copy of the manuscript.

, 6 years after our initial discovery of

the association between PRV and jaundice, there are papers published in three other countries showing the same relationship, one of which is cause and effect (Coho in Japan). We observe this same relationship between jaundice and high loads of PRV

We have done deep sequencing on multiple fish with jaundice and showed that there are no other viruses associated with the disease. Importantly, we now have in situ hybridization showing the localization of PRV within the kidney and liver cells where the necrotic lesions develop. This is very compelling evidence of more than a bystander relationship between this virus and disease in Chinook salmon, and we intend to prepare and submit a paper on this in situ work (done with audit fish, very soon.

Taken together, there is simply too much of a weight of evidence linking this virus with this disease, or very similar looking diseases in other countries in other Pacific salmon species, to dismiss this association,

I thought you should know.

Kisti Miller-Saunders, PhD

Head, Molecular Genetics Pacific Biological Station 3190 Hammond Bay Rd Nanaimo BC V9T 6N7 250-756-7155

Kristi.Saunders@dfo-mpo.gc.ca

s.21(1)(a)

s.21(1)(b)

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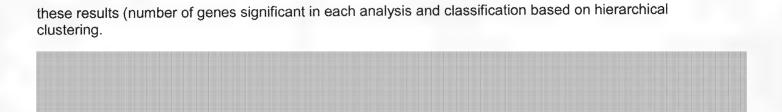
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14(a), 21(1)(b), 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

#### Miller-Saunders, Kristi Miller-Saunders, Kristi From: November-16-17 1:58 PM Sent: To: Taylor, Nathan FW: ACRDP FINAL report Subject: Report\_Creative Salmon ACRDP \_ June 10\_2013\_FINAL.docx Attachments: Here is a copy of the last report I tried to submit, after well over a year of haggling back and forth with various drafts and reanalysing the data multiple times to suite their concerns. In essence, they did not want the inclusion of the PRV data, as they did not consider it "relevant" to the study, Kristi From: Miller-Saunders, Kristi Sent: June-13-13 12:10 PM To: Cc: ; Kaukinen, Karia; Saunders, Mark; Parsons, Jay Subject: ACRDP FINAL report Dear Enclosed is the FINAL revision of the report. I will do no further revisions at this point, and I believe that I have over the past 1+ years. more than dealt with all of the concerns noted by Gary, you The following is a synopsis of what has changed: I have removed the data and information pertaining to wild salmon in this report as it was not part of this study, and has not been validated via a full genome sequence from wild fish. Moreover, I was recently made aware that this report, or this section of the report, was being circulated by someone involved in this project to other individuals within Industry without DFO permission or my knowledge. As this is IP that belongs to the crown and not even to this project, -removing all non-Jaundice "sick" fish (anemia and/or We have re-run the analysis BKD positive) from the jaundice syndrome analysis for both liver and kidney, as well as removing all nonanemia "sick" fish (BKD positive; there were no jaundice only) and fish not scored for anemia from the anemia analysis. We obtained a significant gene list from all four analyses (two tissues, two analyses). We took three approaches to assess whether the signatures from anemia and jaundice alone were correlated: 1) we ran a bootstrap re-sampling correlation analysis on the respective gene lists, and obtained a correlation of >99.9% (i.e. less than 0.1% chance that the gene loadings in these analyses would be due to chance). 2) We assessed the degree of overlap in the top 100, 500 and (for kidney) 1000 genes for each of liver and

kidney. For liver, taking the top 100 significant genes for Anemia (only 523 genes were significant for anemia and 1736 for Jaundice), 96% were also significant for Jaundice, top 500 yielded 86% overlap with Jaundice significant genes. For kidney, top 100 Anemia genes yielded 92% overlap with Jaundice, top 500 was 74%, and top 1000 was 65% (there were 1997 significant genes for Anemia and 2394 for Jaundice), 3) we ran a hierarchical cluster analysis putting all samples not used in the statistical analysis back in, and determined if they clustered similarly when clustering was based on the significant genes in each analysis. We also re-ran the Jaundice/anemia combined analysis—removing the BDK positive fish. We made a new table that shows



As we discussed in the report previously, there are a few "outlier" fish that come out of this analysis—i.e. fish that cluster more intermediately (most notable in Jaundice/anemia combined analysis "a" cluster (1006, 1001, 1004, 1012, 1013), and generally do not do not cluster the same in all analyses (1006, 1001, 1004). You will note that two BKD positive fish clustered more often than not in "A" while two clustered consistently in "B".

I have added this new analysis in, as well as dealing with most of your additional comments.

I will be moving forward with the preparation of a peer reviewed manuscript based on this study, which is the "output" that was originally anticipated.

#### Toisti Miller

Head, Molecular Genetics Section
Pacific Biological Station
Nanaimo, BC
phone (250) 756-7155
fax (250) 756-7053
Please Note new email address effective Jan 2008:
Kristi.Miller@dfo-mpo.gc.ca

#### **ACRDP Final Project Report**

#### **PART I**

- 1. Project #:
- 2. Project Title:

Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon

- 3. Project Duration:
  - 1 April, 2011 31 March, 2012
- 4. Project Leader, contact information:

**Project Manager** 

Karia Kaukinen, MSc Molecular Genetics, Fisheries and Oceans Canada Pacific Biological Station 3190 Hammond Bay Rd Nanaimo, BC V9T 6N7 250 759-8358 karia.kaukinen@dfo-mpo.gc.ca

#### **Project Leader**

Dr. Kristi Miller
Molecular Genetics,
Fisheries and Oceans Canada
Pacific Biological Station
3190 Hammond Bay Rd
Nanaimo, BC V9T 6N7
250-756-7155
Kristi.miller@dfo-mpo.gc.ca

5. Industry partner(s):

Creative Salmon Company Ltd. PO Box 265 Tofino, British Columbia 250-725-2884

s.19(1)

6. Expenditures and variance from budget:

	Contribution	Initial budget	Actual expenditure	Difference
Industry \$	6,000	6,000	6,000	0
Industry (in kind)	16,200	16,200	16,200	0
ACRDP (\$)	72,758	72,758	72,758	0
Other DFO (\$ and in kind)	4,000	4,000	4,000	0
Partners (\$ and in-kind)	1,750	1,750	1,750	0

1.	graduate students etc.):

#### 8. General Comments:

s.20(1)(b)		

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20(1)(b), 20(1)(c)

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#### **PART II**

9. Project rationale (e.g., background information, why solving the problem was of interest to industry, project hypothesis and goals):

A Creative salmon farm-site on the west coast (farm A, Fig 1) has experienced consistent low level mortality with a unique clinical presentation of mild to severe yellow discolouration of the skin (jaundice) and pale gills. The cause has not been identified using standard diagnostic methods, but is hypothesized to be of either viral or environmental toxin origin. The project used a functional genomics approach to elucidate the genes differentially expressed in association with jaundice syndrome. The goal was to increase our understanding of the syndrome. The project also aimed to conduct a thorough epidemiological study to better understand why some farms are more affected and to determine the overall level of mortality attributable to the condition. The ultimate goal was to move closer towards identifying the cause of jaundice that will enable the farms to track, predict, and/or mitigate this syndrome.

10. Short summary of project methods (e.g., experimental and analytical procedures followed, deviations from the originally proposed methods):

Collections were made from two farm sites located on the west coast of Vancouver Island, A and B ,with farm A showing the highest incidence of mortality associated with the jaundice syndrome (see epidemiology) (Fig 1). Moribund or recently dead fish on farm A were collected by divers, and "healthy" swimming reference fish were collected from net pens using hook and line or during harvest in Farm B. From all fish, RNA was extracted from tissue samples of liver, kidney, heart, spleen and gill. The functional genomics study employed a 44K gene oligonucleotide salmonid microarray to identify genes correlated with the jaundice syndrome. Histopathology was done on 15 healthy and 13 freshly dead (less than 12 hours)/sick fish; incorrect preservation of samples collected on April 27, 2011 (6 healthy and 2 sick fish) precluded their analysis by histopathology. RNA was extracted from tissue samples of liver, kidney, heart, spleen and gill. The functional genomics study employed a 44K probe oligonucleotide salmonid microarray to identify genes correlated with the jaundice syndrome.

Thirty-five liver and thirty-six kidney samples were run on the arrays against a reference control containing RNA from all experimental samples and both tissues. The reference control is required to normalize variance in concentration of probes on the array, as well as array to array variability and is thus not meant to represent an experimental sample (i.e. this is different from the "reference" fish that do not show signs of jaundice which were also run individually on arrays and

contrasted with sick fish). After normalization, arrays were analysed statistically using T-tests to identify genes associated with jaundice syndrome, histological lesions associated with the jaundice syndrome, and with high loads of piscine reovirus (see below), and principal components analysis (PCA) was conducted to identify the major physiological trajectories of the data. Functional analyses were performed using Pathway Studio version 9.0.

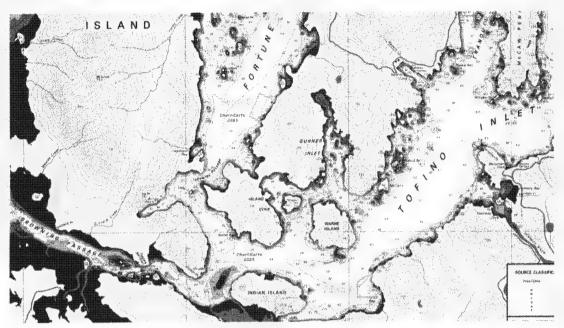


Figure 1. Location of Creative Salmon farm sites.

Quantitative RT-PCR was performed on a subset of host genes in gill tissue aimed at elucidating potential environmental effects (most notably salinity) on gene expression associated with jaundice. Gill tissue was not run on the arrays.

The only deviation from the original plan was the addition of a Fluidigm BioMark scan of infectious agents in liver tissue using published RT-PCR TaqMan assays for 13 infectious agents identified in association with mortality events in salmon and 1 newly identified microbe for which the association with disease is unknown. Correlation analyses were performed with each microbe surveyed to determine if any were associated with the jaundice syndrome. Piscine reovirus (PRV) is the only tested infectious agent that was correlated with the jaundice syndrome. Therefore, additional study was done to validate the PRV results. The other infectious agents were not considered further for this study. Additional study included ABI 7900 RT-PCR validation of PRV in liver, kidney, gill, spleen and heart tissues. Microscopic lesions that occurred with PRV CT's < 26 (indicating higher viral loads) were also identified.

#### 11. Key results (include graphs, data tables, photos, etc. where applicable):

A. Detailed deliverables of project

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20(1)(b), 20(1)(c), 19(1)

of the Access to Information Act de la Loi sur l'accès à l'information

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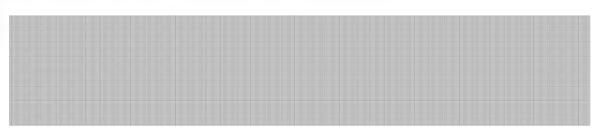
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#### VI. Osmoregulatory assessment of gill tissue



#### VII. References

Chaurushiya, M. S., & Weitzman, M. D. (2009). Viral manipulation of DNA repair and cell cycle checkpoints. *DNA repair*, 8(9), 1166-1176.

Finstad, OW, Falk K, Lovoll M, Evensen, O, Rimstad, R. 2012. Immunohistochemical detection of piscine reovirus (PRV) in hearts of Atlantic salmon coincides with the course of heart and skeletal muscle inflammation (HSMI). *Veterinary Research*. 43: 27.

Forrest JC, Dermody TS. 2003. Reovirus Receptors and Pathogenesis. Journal of Virology 77:9109-9115.

Haller BL, Barkon ML, Vogler GP, Virgin HW 4<sup>th</sup>. 1995. Genetic mapping of reovirus virulence and organ tropism in severe combined immunodeficient mice: organ-specific virulence genes. *Journal of Virology* 69: 357-364.

Garseth AH, Fritsvold, C, Opheim M, Skjerve E, Biering E. 2012. Piscine reovirus (PRV) in wild Atlantic salmon, Salmo salar L., and sea-trout, Salmo trutta L., in Norway. Journal of fish Diseases 36: 483–493. DOI: 10.1111/j.1365-2761.2012.01450

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s.20(1)(b)

s.20(1)(c)

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Mbisa JL. 2002. Investigation of the virus-host cell interactions involved in reovirus inclusion formation. PhD Dissertation, University of Ottawa (https://www.ruor.uottawa.ca/fr/handle/10393/6267)

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Wiik-Nielsen CR, Løvoll M, Sandlund N, et al. 2012. First detection of piscine reovirus (PRV) in marine fish species. *Diseases of Aquatic Organisms*. 97:255-258.

#### VIII. Recommendations to industry on next steps

- Laboratory challenge study will cell free size-filtered lysates to establish whether the piscine reovirus can cause signs of jaundice in BC Chinook salmon.
- Include other diagnostic testing such as hematocrit, blood assessment to better understand the cause of the anemia associated with the syndrome
- Better define risk factors contributing to this syndrome

## 12. Resulting key improvements to sustainable aquaculture and scientific advancements:

- Assessment of a potentially powerful novel diagnostic tool genomic characterization as a diagnostic tool for fish health
- Illustrating a multidisciplinary approach (genomics, standard veterinary diagnostic techniques, histopathology, epidemiology) in attempt to solve fish health issues

#### 13. Suggested next steps, future research/development/innovation needs:

 Conduct laboratory studies to assess the role of PRV load and the potential to elicit jaundice presentation or disease.

- If PRV is shown in follow-up studies to be causative of the Jaundice syndrome.
  - Routine monitoring of PRV and biomarkers for disease could enable more precise tracking of the virus and disease progression.
     Biomarkers alone could be useful if PRV is not causative.
- Whole genome sequencing of nucleic acids (DNA and RNA) of affected fish to gain the full sequence of PRV in BC
- Phylogenetic analysis of the full sequence of the piscine reovirus here in BC to determine its relationship with European strains.
- 14. Copies of publications, reports or articles produced in reference to the project:

N/A

- 15. Identify any invention or innovation that may have resulted from this Project, including any new process or technique.
  - High throughput microbe screening on the Fluidigm BioMark system was developed and applied during the course of this study, although it was not principally motivated or financed by this study.

# PART III Declaration: I \_\_\_\_\_\_\_\_ have completed the report and declare that to the best of my knowledge the report is accurate. Signature Date Approved by: DFO Project Authority Date

Date

Industry Project Authority

#### Miller-Saunders, Kristi

From:

Taylor, Nathan

Sent:

November-16-17 10:10 AM

To:

Miller-Saunders, Kristi

Subject:

unapproved ACRDP report

Hi Kristi,

Do you have a copy of the draft Creative Salmon report that collaborators would not sign off on?

N.

Nathan G. Taylor, Ph.D.

Division Manager | Directeur de secteur

Aquatic Diagnostics Genomics and Technology Division | Division des diagnostics, la genomique, de la technologie aquatique

Fisheries and Oceans Canada | Peches et Oceans Canada Pacific Biological Station | Station biologique du Pacifique 250-756-7395

#### Dickie, Catherine

From:

Moore, Wayne

Sent:

November 16, 2017 10:21 AM

To:

Taylor, Nathan; Lowe, Carmel

Subject:

Re: URGENT

I sent to Carmel all we could find. My understanding is that prior to 2013 the agmts were held in the region and Brenda might be your best contact.

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Taylor, Nathan

Sent: Thursday, November 16, 2017 1:18 PM

To: Moore, Wayne; Lowe, Carmel

Subject: RE: URGENT

Hey Wayne – did you have any luck finding the Collaborative Agreement for the project in the system? I've been looking on my end as well with no luck.

N.

From: Moore, Wayne

Sent: Wednesday, October 25, 2017 5:03 PM

To: Lowe, Carmel Cc: Taylor, Nathan Subject: RE: URGENT

This is the project I think...trying to find an e-copy of agreement in system.

Photo: Mike Foreman (DFO)

## Genomic characterization of jaundice-associated mortality events in cultured Chinook Salmon

This project was undertaken to determine whether a jaundice syndrome associated with low-level mortality in Chinook Salmon farmed in Tofino Inlet was more likely caused by a viral infection or an environmental toxin. Our project combined genomics, histopathology, epidemiology, and standard veterinary diagnostic techniques to determine which of these etiologies was more likely involved. Prevalence of jaundice syndrome was consistently greater at farm A than B over multiple years. The most significant lesions included tissue necrosis and fibrin deposition, primarily in kidney and liver. Genomic signatures comprised of thousands of differentially regulated genes occurred in both kidney and liver, with strong effects on immune response, proteolysis, metabolism, and cell cycle. The types of immune processes elicited were highly consistent with a viral etiology (response to virus, response to exogenous dsRNA, Stat signaling, type-I interferon response, viral replication); conversely, there was no signal that could be construed as toxicant-response. Based on a PCR survey of infectious agents, fish with jaundice syndrome commonly had greater loads of piscine reovirus than did healthy fish. This virus is purported to cause heart and skeletal muscle inflammation (HSMI) in Atlantic Salmon in Europe, but the lesions associated with HSMI are very different from lesions in Chinook Salmon with jaundice syndrome. Tissue tropism is not uncommon with reovirus infections, so it is possible that this virus could affect different tissues in different species. As a whole, this research

supports a viral etiology, however, more research will be required to determine if the piscine reovirus is causative of, associated with, or merely a bystander to the jaundice syndrome.

apr. 2011 - apr. 2012

Funded by: DFO - Aquaculture Collaborative Research and Development Program (ACRDP) co-

funded by: Creative Salmon

project lead: Kristi Miller (DFO)

Project team: Karia Kaukinen, Brad Davis (DFO); (CAHS)

collaborators: Gary Marty (BC Ministry of Agriculture)

Contact: Kristi Miller@dfo-mpo.gc.ca

From: Lowe, Carmel

Sent: October 25, 2017 7:24 PM

To: Moore, Wayne < Wayne Moore@dfo-mpo.gc.ca > Cc: Taylor, Nathan < Nathan Taylor@dfo-mpo.gc.ca >

Subject: RE: URGENT

KMS

Cornel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.gc.ca
Telephone | Téléphone 250-756-7177
Facsimile | Télécopieur 250-729-8360

Government of Canada | Gouvernement du Canada

From: Moore, Wayne

Sent: Wednesday, October 25, 2017 4:23 PM
To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>
Cc: Taylor, Nathan < Nathan.Taylor@dfo-mpo.gc.ca>

Subject: Re: URGENT

Will try to find. it has been a few years. Who is PI?

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Lowe, Carmel

Sent: Wednesday, October 25, 2017 7:20 PM

To: Moore, Wayne Cc: Taylor, Nathan Subject: URGENT

Wayne,

Can you send Nathan and I a copy of the ACRDP proposal that relates to the hot issue of the day? Neither Nathan nor I have a copy of it and the PI is on the C3 boat.... s.19(1)

#### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.gc.ca
Telephone | Téléphone 250-756-7177

Facsimile | Télécopieur 250-729-8360

Government of Canada | Gouvernement du Canada

#### McLeod, Patricia

From:

Miller-Saunders, Kristi

Sent:

November 16, 2017 2:01 PM

To:

Taylor, Nathan

Subject:

FW: Creative salmon report

Response from Jay back in 2013

From: Parsons, Jay Sent: July-08-13 6:45 AM

**To:** Miller-Saunders, Kristi; Saunders, Mark **Subject:** RE: Creative salmon report

Kristi,

I will follow-up and request a timeline on when he will provide comments and ask if a follow-up teleconference call needs to be organised.

Mark are you in support of this approach?

Thanks, Jay

#### Jav Parsons, PhD

Director | Directeur
Aquaculture Science Branch | Direction des sciences de l'aquaculture
Fisheries and Oceans Canada | Pêches et Océans Canada
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6
tel. | tél. 613-990-0278 fax. | téléc. 613-990-0313
email | courriel Jay.Parsons@dfo-mpo.gc.ca

**From:** Miller-Saunders, Kristi **Sent:** July-04-13 3:58 PM

**To:** Saunders, Mark; Parsons, Jay **Subject:** Creative salmon report

s.19(1)

I sent this report back to Creativ	ve salmon in the middle of June and I cc'd both of you at that time. As I had not heard
anything back, I called	who said that they were going to come back with comments and revisions again,
but that was handling this.	In my email, I made it clear that we were done with revisions.
	This work needs to move to publication,

Thanks,

Kristi Miller

Head, Molecular Genetics Section
Pacific Biological Station
Nanaimo, BC
phone (250) 756-7155
fax (250) 756-7053
Please Note new email address effective Jan 2008:
Kristi.Miller@dfo-mpo.gc.ca

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s.21(1)(a)

s.21(1)(b)

Parsons, Jay	
From:	- Creative Salmon -
Sent:	Friday, November 17, 2017 11:30 AM
To:	Parsons, Jay; - Creative Salmon
Subject:	RE: call to discuss ACRDP manuscript
Attachments:	RE: call to discuss ACRDP manuscript; RE: call to discuss ACRDP manuscript
Hi Jay, Yes definitely, a good ide Talk next week,	ea to include Nathan as well. He has already accepted the meeting invitation.
Sent: Friday, November To: Creat Subject: RE: call to disc	tive Salmon; - Creative Salmon
Original Appointmen	
	reative Salmon
Sent: Monday, November	
	tive Salmon; Parsons, Jay; Marty, Gary D AGRI:EX - Creative Salmon
	ber 21, 2017 9:00 AM-9:30 AM (UTC-08:00) Pacific Time (US & Canada).
Please call:	
Participant Code:	

s.16(2)(c)

s.19(1)

## Pages 303 to / à 1623 are withheld pursuant to section sont retenues en vertu de l'article

23

of the Access to Information Act de la Loi sur l'accès à l'information

#### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

November-23-17 4:52 PM

To:

Lowe, Carmel

Subject:

BCSFA meeting information, As requested

#### BC Salmon Farmers Association Workshop Exploring PRv and HSMI in Europe and B.C.

On November 27-28th, 2017, the BC Salmon Farmers association, in collaboration with the BC Center for Aquatic Health Sciences, is hosting a workshop in Campbell River to explore the state of the science on PRV and the disease Heart and Skeletal Muscle Inflammation (HSMI). In addition to a strong representation of scientists from Fisheries and Oceans Canada, they have invited speakers from Norway (academics and industry scientists), the US Geological Service, University of BC, and the BC Center of Aquatic Health Services. The workshop will be moderated by George Iwama from Quest University and Gary Marty from the BC Ministry of Agriculture. Presentations will provide an overview of the virus and disease manifestation in Norway and the Atlantic and Pacific coasts of Canada in Atlantic and Pacific salmon. The Norwegian scientists will also touch on mitigation measures being put in place in Norway to tackle this PRV-related disease. Given there has been strong debate amongst researchers in BC over the risk that PRV poses to wild salmon, this meeting provides an important venue for the key researchers and industry to come together and forge a path forward to understand the knowledge gaps in our understanding of impacts and risks associated with PRV in BC salmon.

Kristi Miller-Saunders, PhD

Head, Molecular Genetics
Pacific Biological Station
3190 Hammond Bay Rd
Nanaimo BC V9T 6N7
250-756-7155
Kristi.Saunders@dfo-mpo.gc.ca

#### Gheorghe, Tricia

From:

Gheorghe, Tricia

Sent:

November-23-17 2:47 PM

To:

Parsons, Jay

Subject:

RE: acrdp proposal

Attachments:

P-11-02-007 Proposal.pdf; P-11-02-007 Report Final.doc; P-11-02-007 CA Signed.pdf

Included the proposal, final report, and signed CA.

Cheers!

From: Parsons, Jay

Sent: November-23-17 2:31 PM

**To:** Gheorghe, Tricia **Subject:** acrdp proposal

Tricia, could you please resend me the acrdp proposal from pacific region with creative salmon from a few years ago

again. Thanks, Jay



DFO-ACRDP Use only

Fisheries and Oceans Çanada

1. INDIVIDUAL OR	ORGANIZAT		ON CON	X (V)	***************************************		
INDIVIDUAL OR ORGANIZA Creative Salmon Ltd		IN OPERATION SINCE 1990		INCORPORATED  D FED			
ADDRESS PO Box 265			CTTY Tofino	PROV BC		VCE	POSTAL CODE VOR 2Z0
TELEPHONE # 250 725 2884	FAX # 250 72	5 2885	F-MAIL7	ADDRESS			adhanan e e e e e e e e e e e e e e e e e e
HEAD OFFICE ADDRESS (IF I	NOT SAME)		СПУ		PROVIN	CE.	POSTAL CODE
Number of employees:	1-4	5-19	20-49	50-100	X.	1004	-
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PROJECT MANAGER CONTACT (NAME)  Karia Kaukinen  TELEPHONE #  250-759-835			FAX#			E-MAIL Karia.kaukinen@dfo-mpo.gc.ca	
2. PROJECT INFORMATION Project Title: Genomic ch		of jaundice-associa	ted mortal	ity events in cult	ured Chi	nook salmo	)n
A. The following should be     A proposal giving the c     Budget details (provide     Project budget summar	letails of the pro details of each	eject as outlined on the line item)	ie Proposal			**************************************	
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Fisheries and Oceans Canada Pêches et Océans Canada

#### Aquaculture Collaborative Research and Development Program (ACRDP)

#### APPLICATION FORM

1. INDIVIDUAL OR ORG	ANIZATI	ON INFORMATI	ON					
individual or organization full legal name Creative Salmon Ltd				IN OPERATION SINCE 1990		INCORPORATED  □ FED ⊠PROV □N/A		
ADDRESS PO Box 265		CITY Tofino		PROVE BC	(CE	POSTAL CODE VOR 2ZO		
TELEPHONE # FAX # 250 725 2884 250 725 2885			E-MAIL	ADDRESS				
HEAD OFFICE ADDRESS (IF NOT	SAME)		CITY		PROVIN	CE	POSTAL CODE	
Number of employees: 1-4	â	5-19	20-49	50-	100X_	1004	*	
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PROJECT MANAGER CONTACT (NAME)  Karia Kaukinen  TELEPHONE #  250-759-835						B-MAIL Karia.kau	E-MAIL. Karia.kaukinen@dfo-mpo.gc.ca	
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C. Other sources of funds related 1.		nal priority <b>Indust</b> nject (if applicable, p				•		
hereby declare that all of the abotherwise, I agree to withdraw my								
roject proponent Signature	Please Pri	nt Name <u>CLEARLY</u>	Title			Date		
DFO Project Lead Signature	Please Pri	nt Name <u>CLEARLY</u>	Emai	l address		Date		
OFO-ACRDP Use only	Project ID	Number		Submitted	. 2 49. 49. 2 2 22. 4	Region		

Canada

# Aquaculture Collaborative Research and Development Program (ACRDP) PROPOSAL GUIDELINES

Please submit a proposal giving the following details:

- 1. Project title: Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon
- 2. Name, address and position of project manager

Karia Kaukinen Bi-02 in the Molecular Genetics Laboratory Pacific Biological Station 3190 Hammond Bay Rd Nanaimo, BC V9T 6N7 250-759-8358

3. Description of project work team and required qualifications for key positions (with names, addresses, titles, and CV's where available; maximum length 4 pages per team member)

Kristi Miller, PhD Head, Molecular Genetics, PBS Genomics lead, genomics data analysis and interpretation

Karia Kaukinen, MSc BI-02 Molecular Genetics, PBS Project manager, Genomics laboratory research, genomics data analysis

Center for Aquatic Animal Health Sciences 871A Island Highway, Campbell River BC V9W 5B1 Fish Health expertise, industry liaison, epidemiology expertise

- Creative Salmon
Tofino BC

Industry Partner

4. Project problem / rationale (maximum length ½ page)

Over the past seven years mortalities of Chinook salmon farmed in the Tofino inlet have been observed with unique clinical presentation. The salmon present with mild to severe yellow discolouration of the skin (jaundice). This is most evident on the abdomen and around the eyes. These fish also have very pale gills indicating anemia. Internal signs include pale livers and often the stomachs of the fish are empty indicating the fish have not eaten for a number of days

although the overall condition of the fish is good. Grossly the other organs appear unaffected. The clinical presentation is very different from Marine Anemia syndrome, another Chinook salmon disease, which typically presents with splenomegaly, renomegaly, and anemia (Kent and Poppe 1998).

Histological examination has found severe liver and kidney damage (hydropic degeneration). The proposed etiology includes a pathogen or exposure to a negative environmental influence (hereafter referred to as an undefined toxin). Repeated testing using traditional diagnostic tests have been unable to identify a pathogen. Tests including classical bacterial culture, viral cell culture, PCR, blood assessment and histopathology have yielded negative results for pathogens including *Renibacterium salmoninarum* (BKD), *Listinella sp* (vibriosis), VHSV, IHNV, ISAV, VEN, EIBS, Loma salmonae, and *Nucleospora salmonis* (marine anemia).

Little is known of the epidemiology of the condition. It affects fish that have been in sea water for greater than 6 months and therefore is not considered related to smolt quality. There appears to be a seasonal pattern to this condition with clinical signs and mortalities observed late fall/early winter (December) spiking in the winter and apparently resolving by early summer. This condition is most typically observed at the farm site operated by Creative Salmon that contains the greatest freshwater influence, which is one reason to suspect an environmental effect may be at play, although it is sometimes observed at relatively lower incidence levels at other farm sites. It has been seen in most of the generations stocked at the freshwater-influenced farm even though the company operates single year class sites with a fallow period before re-stocking. At the most affected farm most often one or two pens of fish are severely affected, however the condition may be seen in many of the pens. The mortalities levels in most heavily affected pens typically would be several folds higher than the other pens on the farm. For example in January 2011, the single affected pen (of an 8 pen site) disproportionately made up 35% of the mortalities grossly examined. Of the mortalities examined from this single pen over 77% of the fish examined exhibited jaundice. Total mortality attributed to this condition has not been fully assessed although at certain times of the year it can be as significant as other diseases.

Currently there are no tools available to manage the problem. A better understanding of the epidemiology and etiology would enable us to develop these tools.

#### 5. Project objectives (maximum length ½ page)

Our main objective is to apply functional genomics technology to gain a better understanding of the factors that may underlie the poorly understood jaundice-related disease experienced by farmed Chinook salmon in Tofino. Of most import, Creative Salmon managers need to know whether this disease is more likely the result of an infectious agent or environmental conditions. If the genomic signature indicates that an infective agent is likely involved, they will pursue the identification of this agent through 454 sequencing of affected tissues in a follow up study. If the genomic signature is more likely associated with environmental conditions at this farm site, e.g. low salinity, toxicants, or other factors, follow-up studies could be performed to assess the most likely environmental mechanisms. In either case, knowledge of the mechanisms leading to this disease will potentially provide managers with tools to track, predict, and/or potentially mitigate the impact of this disease in future.

Our other objective is to improve our understanding of the epidemiology of the condition — why is the condition more prevalent at one farm as compared to the others in the same area, or in some pens and not others? This would include examining the mortality pattern, and determining overall mortality attributed the condition, and the environmental factors associated with it.

## 6. Description of work and experimental protocol (maximum length 2 pages)

Fish on Creative Salmon's farms are regularly screened for health, and the presence of jaundice is one of the metrics that is tracked bi-weekly in each netpen at each site. While mild jaundice can occur to some degree at all sites, jaundice-related mortality is predominant at the farm with the greatest freshwater influence. Despite extensive histological study, we still do not understand whether the jaundice may result from an infective agent or may be a manifestation of environmental conditions, possibly relating to low salinities, in the winter, or a combination of both. Hence, we need to design an experiment that considers both possibilities.

The molecular genetics laboratory has successfully used microarray approaches on wild fish to assess unknown factors associated with poor performance (e.g. Miller et al. 2010) and transcriptional responses to shifting environments (Miller et al. 2009; Evans et al. In Review). We have also conducted microarray studies of host response to disease (Miller et al. 2007; IHNV) and have conducted studies with Peter Ross at IOS on the influence of toxicant exposure on immune response to Vibrio (data still in preparation for publication). Moreover, we have been working closely with a bioinformatics group at UBC, lead by Dr. Paul Pavlidis, on the development of meta-analysis tools to identify correlated profiles among microarray experiments. Hence, we have the experience and expertise to undertake this exploratory study. We will use similar experimental designs and approaches as we have used in previous studies (e.g. Miller et al. 2009) whereby we apply balanced replication across each biological variable, or treatment, of interest. Here, treatments are not really treatments, as one would define in a controlled laboratory study, but rather biologically meaningful entities, like sample sites, disease state, and life-history stage.

Approach: If an infective agent is involved, it is important to determine whether it is present in smolts used to stock the farm sites, or whether it likely emanates from the marine environment. As such, our study will include smolts used to stock net pens in 2011 (Treatment 1; note that the sample size of smolts would only detect positives that affected 10% or more of fish, so it is not an exhaustive, definitive assessment of the role of freshwater). To control for the environment, we will contrast apparently healthy (no evidence of jaundice) fish a farm that is not substantively affected by jaundice (Treatment 2) with healthiest fish that can be obtained sampled from the affected freshwater-influenced farm (Treatment 3). To obtain healthy fish, we will chum fish from netpens that have been shown to have the lowest levels of jaundice and conduct histological analyses to ascertain their state of health. Fish that were collected in this manner but deemed to be positive for, but not dying of, jaundice at the time of collection will also be included in the analysis to control for the effects of morbidity (Treatment 4). Finally, moribund fish with clear evidence of jaundice will be included in the study (Treatment 5). Multiple tissues from all collected fish will be examined histologically.

Head and Posterior kidney, spleen, liver, heart, muscle, gill, and blood of 10-15 fish will be collected for each "treatment" category.

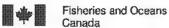
- I. Smolts
- II. Healthy fish from unaffected farm at mouth of the inlet
- III. Healthy fish from affected farm
- IV. Fish with histological signature of jaundice but not moribund at affected farm
- V. Moribund fish with histological signature of jaundice at affected farm

In anticipation of this study, some of the samples of moribund and jaundiced fish were collected during a mortality event at the end of February 2011. Additional samples of jaundiced but not moribund and healthy fish from both farm sites will be collected in early March, along with additional moribund and jaundiced fish, if still present. Smolts will be collected in April before they are put to sea.

The microarray study will be performed on cDNA from liver tissue, as liver is one of the most severely affected organs and is also the primary tissue for detoxification. We will keep the remaining tissues for potential future study. In a balanced experimental design, twelve biological replicates (individuals) will be included for each of the five treatments, with the total study comprising 60 arrays. The Salmonid Agilent 4x44K olignucleotide arrays developed through the cGRASP program by Ben Koop's laboratory at University of Victoria will be used in the study, with approaches similar to those we have used in other studies (e.g. Miller et al 2009, 2011). A reference sample comprised of liver cDNA from all of the individuals used in the study (labeled with Alexa555) will be hybridized along with the experimental sample (labeled with Alexa647) on each array. After slide quality assessment and Lowess normalization, arrays will be statistically analysed using both supervised (multifactorial ANOVA with posthoc testing via Mann Whitney U t-test) and unsupervised (Principle Component Analysis) approaches to identify transcriptional differences among treatments and the main physiological trajectories in the data. Functional analysis of the biological processes over-represented among the differentially regulated genes will be determined using the programs DAVID (http://niaid.abcc.ncifcrf.gov/) and PathwayStudio (Ariadne Genomics). Further information on the functional role of the most significantly differentially regulated genes will be gleaned from the protein literature mining website ihop (http://www.ihop-net.org/UniPub/iHOP/).

Many salmon diseases have already been characterized by microarrays (e.g. Miller et al. 2007, Rise et al. 2004, Morrison et al. 2006, Baerwald et al. 2008), including the infectious diseases ISAV, IHNV, VHSV, Aeromonas salmonicida, Amoebic gill disease, salmon louse, and others. A wealth of microarray studies also exist for toxicant responses in fish (e.g. Finn et al. 2007, Tilton et al. 2006, Hook et al. 2006) for a diverse array of chemicals, including PBDE (flame retardant), endocrine disrupting compounds, heavy metals, PCB's, and others. In our own research, we also have data from both wild fish and controlled laboratory studies that assesses responses to shifting salinities and temperature, and we have assessed wild salmon in the ocean at the same time of the year as the fish from this study (the first winter at sea), some even from the west coast. We will use these studies as a backdrop to assess the possible correlation of signatures emanating from jaundiced Chinook salmon with signatures associated with pathogenic disease, toxicant exposure and other environmental stressors. To do this, we will use the recently develop meta-analysis program for microarray data GEMMA

Canada



(<a href="http://www.chibi.ubc.ca/maintenance.html">http://www.chibi.ubc.ca/maintenance.html</a>), developed from our colleague and collaborator on our wild salmon studies, Paul Pavlidis. The literature mining software available within PathwayStudio will be similarly applied.

Because the farm site most affected by the jaundice-related disease also contains the lowest salinities of all the farm sites, we will additionally address the potential role that osmoregulatory dysfunction may play in the manifestation of this disease. We will assess the osmoregulatory state of fish in each treatment through quantitative PCR of gill cDNA for the isoforms of Na<sup>+</sup> K<sup>+</sup>-ATPase, cold-inducible RNA binding protein, prolactin, and growth hormone. We will also determine osmolality and ion concentrations in blood plasma, which will indicate whether fish are able to maintain homeostasis in their gills.

We will evaluate health records collected and environmental data collected for the last seven years for the farm where the jaundice is most prevalent. Similar data will also be collected from one other farm where jaundice has not been observed or has been observed at very low prevalence. Health data is collected twice a week over the duration that the fish are at sea (~18-20mos). Environmental data (temperature, salinity and dissolved oxygen) is collected on a daily basis. The data will encompass 3 different generations stocked on the farm. These records will be used to estimate prevalence and describe the pattern of the disease both temporally (i.e. time of onset, age of onset, duration of disease, environmental profile) and spatially (difference in prevalence between years, between pens, between farm sites). This will be the first epidemiological analysis of this condition.

#### References:

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  Transcriptomis of environmental acclimation and survival in wild adult Pacific sockeye salmon (Oncorhynchus nerka) during spawning migration. Molecular Ecology: In Review.
- Finne EF, Cooper GA, Koop BF, Hylland K, Tollefsen KE. 2007. Toxicogenomic responses in rainbow trout (Oncorhynchus. *Aquatic Toxicology* 81:293-303.
- Hook, SE. AD Skillman, JS Small, IR Schultz. 2006. Gene expression patterns in rainbow trout, Oncorhynchus mykiss, exposed to a suite of model toxicants. *Aquatic Toxicology* 77: 372-385.
- Kent, M.L. and T.T. Poppe. Diseases of seawater netpen-reared salmonids Pacific Biological Station Press, Nanaimo, British Columbia, Canada (1998:) 138 pp.
- Miller, K.M., Li, S, Kaukinen, K.H., Ginther, N., Hammill, B., Curtis, J.M.R., Patterson, D.A., Sierocinski, T., Donnison, L., Paylidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., and Farrell, A.P. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science: 331: 214-218.
- Miller, KM, AD Schulze, N Ginther, S Li, DA Patterson, AP Farrell, SG Hinch. 2009. Salmon Spawning Migration: Metabolic Shifts and Environmental Triggers. Comp. Biochem Physiol D 4: 75-89.
- Miller, K.M., G. Traxler, K.H. Kaukinen, S. Li, J. Richard and N. Ginther. 2007. A cDNA microarray study of Atlantic salmon (Salmo salar) response to Infectious Hematopoietic Necrosis (IHN) virus. Aquaculture 272 (Supplement 1): S217-S237.
- Morrison RN, Cooper Ga, Koop BF, et al. 2006Transcriptome profiling the gills of amoebic gill disease (AGD)-affected Atlantic salmon (Salmo salar L.): a role for tumor suppressor p53 in AGD pathogenesis? Physiological genomics 26(1):15-34.

- Rise ML, Jones SR, Brown GD, et al. 2004Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage and hematopoietic kidney response to Piscirickettsia salmonis infection. *Physiological genomics* 20(1):21-35.
- Tilton\* SC, Givan† Sa, Pereira‡,§ CB, Bailey\*,‡ GS, Williams\*,‡ DE. 2006Toxicogenomic profiling of the hepatic tumor promoters indole-3-carbinol, 17beta-estradiol and beta-naphthoflavone in rainbow trout. Toxicological 90(1):61-72.
- 7. Description of how this project meets the goals, objectives and priorities of the program (maximum length 1 page)

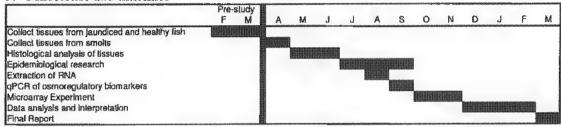
This project meets three of the four goals of the ACRDP program. It provides improved competitiveness of the Canadian aquaculture industry by providing scientific information that may be used to manage or mitigate disease impacts on aquaculture production. It provides increased collaboration between the department and industry on scientific research and development by using genomic technology and expertise available within the department to help resolve issues impacting the industry. It also increases the scientific capacity for essential aquaculture research by employing a technology that has yet to be fully realized by the salmon farming community in BC.

The research project addresses priority research within two of the broad research and development objectives: Optimal fish health and Industry environmental performance. By assessing the potential involvement of pathogen-driven versus environmentally-induced factors in the jaundice-associated disease, the project assesses the potential role of biological causative agents associated with disease and the influence of the environment. Moreover the findings of this project could impact health management strategies and lead to better tools for disease surveillance and detection.

- 8. Detailed deliverables of project (must include final project report)
  - I. Collections of tissues from jaundiced and healthy fish that can be used for transcriptional studies and pathogen isolation and sequencing in future
  - II. Full functional genomics assessment of jaundiced fish, including the lists of genes and biological processes differentially regulated in response to the jaundice-associated disease and an assessment of the potential roles of pathogens versus environmental perturbations in eliciting the disease.
  - III. A list of genes that might be useful biomarkers to predict disease and stage disease progression
  - IV. Characterization of osmoregulatory state of fish at the two farm-sites through biomarker screening of gill tissue and plasma ion and osmolality levels
  - V. Epidemiological analysis of the disease prevalence at each of the farm sites over the past 7 years and environmental data
  - VI. Recommendations to industry on next step research to either identify an infective agent associated with the disease or to narrow down potential environmental factors involved.

- VII. Manuscript to be published in a peer-reviewed journal describing results (will not be complete until after the project ends)
- VIII. Final project report to ACRDP





#### 10. Organisation profile (maximum length ½ page)

Creative Salmon, established in 1990, is one of only a few premium growers of Chinook salmon in Canada and the world. The company's six saltwater tenures are located in the traditional territory of the Tla-o-qui-aht First Nations. Creative Salmon and its staff are active and enthusiastic community partners.

Creative Salmon has a committed and dedicated workforce of approximately 55 full time staff – and these employees are crucial to the company's continued success. The company bases its production management on organic principles, including: a low density, low-stress growing environment that encourages fish health and welfare; close contact between staff fish culturists/farmers and the fish they raise; and husbandry practices and standards that grow healthy, high quality salmon with a small environmental footprint.

Creative Salmon produces over per annum by operating an integrated production cycle. With painstaking control of quality from egg to harvest, the company has established an enviable reputation for high quality Chinook salmon, serving the most discerning clientele throughout Canada, the USA and Japan.

# 11. Partner(s) profile, including contact name and information (if applicable) (maximum length ½ page)

The BC Centre for Aquatic Health Sciences (BC CAHS) is a research facility designed to fill the void in marine health research capacity in British Columbia. It was established in 2005 in Campbell River BC. BC CAHS exists to advance understanding of British Columbia's aquatic resources by addressing issues of aquatic animal health and welfare, production and aquatic food safety, thereby facilitating the economic, social and environmental sustainability of British Columbia's aquatic based resource industries and increasing research and service capacity in rural and coastal communities. The strength of the facility has been the success that BC CAHS researchers have had in liaising with the appropriate government and academic researchers to facilitate research important for the environment and the continued sustainability of our aquatic based resource industries.

Canada

In addition to being the operates a private practice and has been providing veterinary services to Creative Salmon for the last 4 years.

Estimated budget – provide details of each budget item, a budget summary for each fiscal year, if applicable, and a total project budget summary.

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# Aquaculture Collaborative Research and Development Program (ACRDP) Budget Summary by Fiscal Year\_\_2011\_\_\_\_\_\_(1 April - 31 March)

Please provide a budget for the total project with each fiscal year detailed on separate attached sheets. Details for each of the line items should also be documented on separate sheets.

Description	Industry Cash Contribution	Industry In- kind Contribution	ACRDP Contribution	DFO In-kind Contribution	Partner In- kind Contribution <sup>1</sup>	
						Total
Salary	•			4.000		4 000
Scientist-Millar				4,000		4,000
Veterinarian-						
Biologist						
Technicians/Biologist						
Post-Doc / Students						0
Divers		5,000				5,000
Sub-total						0
Equipment						0
Computer Equipment						0
Lab Equipment						0
Field Equipment						0
Other						0
Sub-Total						0
Material and Supplies						0
Lab		1,000	29,120			30,120
Field			510		150	460
Publication costs			500			400
Healthy Fish		1,500				0
Sub-Total						0
Travel						0
Field		2,100				2,100
Meetings						
Conferences					•	0
Other						0
Sub-Total						0
Other						0
Administrative						0
Facilities						0
Other expenses						0
Sub-Total						0
Grand Total		16,200	72,758	4,000		95,280
% OF		. wys. ~ 57	,	.,,		
CONTRIBUTIONS	0.082	0.223				0.305

#### Details of expenditures:

#### Salaries

Salary for ½ year of a BI-02 (Karia Kaukinen) in the molecular genetics laboratory who will be responsible for project management and reporting, RNA extractions, biomarker qPCR, microarray experiment, and preliminary data analysis provided by ACRDP. Karia's salary is

The industry will provide a cash contribution for of these salary dollars.

Dr. Miller will dedicate of her time to the project, at an in kind cost of \$4,000. Most of this time will be spent on data analysis and interpretation.

Fish Health Technician ( to provide assistance in field - 4 days @ = and Biologist ( will be involved in data assembly for the epidemiological evaluation -3 days @ to assist in data collection. These people are employed by Creative Salmon and their time will be provided as in-kind contribution.

Total = \$2,150

Commercial Contract Divers will be used to collect samples 5 days @ \$1000/day = \$5000. This will provided as an in-kind contribution from Creative Salmon

# Equipment

## <u>Lab</u>

Technology platforms and lab infrastructure are already in place in the DFO Molecular Genetics Laboratory (MGL) at the Pacific Biological Station for experimental microarray research. Dr. Miller is the Head of this laboratory, and in 2004, began developing a functional genomics laboratory for gene expression research. Current infrastructure for functional genomics research includes: Retsch MM301 mixer mill, Beckman Biomek NX<sup>P</sup> Robot with a Span-8 Head and Integrated DTX 880 Plate Reader, TECAN HS 4800 Pro Hybridization Station with two extension units (24 slides/day capability and potential to expand to 4 extension units with 48 slide/day capability), Packard BioScience ScanArray Express Microarray Scanner, MJ Research PTC 100 PCR machines, Millipore MilliQ Biocel Water Purification System, ABI 7900HT Fast Real-Time PCR System and Integrated Carousel (384 well plate platform). The MGL is built for high throughput experimental research and application, and easily contains the capacity to carry out the proposed study. No funding is being requested for use of this equipment.

<u>Field</u>

Canada

Field supplies including formalin, histology cassettes, tools (forcep, scalpels, scissors etc) will be provided in kind by BC CAHS - value \$150

Blood collection materials and RNAlater \$510

**Materials and Supplies** 

Creative salmon is donating 30 healthy fish to the project at a market value of \$1,500. As the brains will be removed, these fish will not be saleable.

#### Lab

Histology 75\*\$32/sample = \$2,400 Creative salmon will provide \$1,000 as an in-kind contribution.

Plasma ion and osmolality will be conducted in David Patterson's lab. 60 samples \* \$12/sample = \$720

Biomarker study 60 fish \* 10 biomarkers (including housekeeping genes) \* \$5/biomarker = \$3,000

Microarray Study: Each array costs a total (excluding labour) of \$400 to run, which includes the slide costs (\$160 per array within the 44K slide), RNA extractions, amplification, labeling, and hybridization costs, as well as a portion of the service contract on the Tecan hybridization robot and the Tecan robotic slide reader. There is also a built in 5% margin to accommodate slides that have to be re-run. Microarray study 60 fish \* 400/array = \$24,000

#### **Publication costs**

\$500 will cover the cost of publishing a manuscript with one colour figure.

#### Travel

Collaborative Meetings travel CAHS-DFO \$400 for 5 trips/year
Field Travel CAHS-Tofino for sample collections \$1500 for 4 trips (600km @\$.50/km per trip +
accommodation (if required)+food), industry in-kind contribution
Boat Travel for sample collection \$600 for 4 trips (\$150/day), industry in-kind contribution.

1. If more than one partner, please provide details of contribution from each one.

Canada

P11-02-007

July 5th, 2011

K. Kaukinen

Judy Volk

# DFO-51510-841-121-53248 CANADA - Creative Salmon Ltd. 51510-841-760-57440 COLLABORATIVE AGREEMENT

THIS AGREEMENT is made in duplicate as of June 6<sup>th</sup>, 2011.

Between:

HER MAJESTY the Queen in right of Canada ("Canada"), as represented by the

Minister of Fisheries and Oceans on behalf of Science in the Pacific Region ("DFO").

And:

Creative Salmon Ltd., a corporation incorporated under the laws of British Columbia, with a head office located at PO Box 265 Tofino in the province of British Columbia

(the "Organization").

#### RECITALS

WHEREAS Canada and the Organization (each shall be referred to as "Party" and together they shall be referred to as "Parties") wish to collaborate under the Aquaculture Collaborative Research and Development Program (ACRDP) on the research project "Genomic characterization of jaundiceassociated mortality events in cultured Chinook salmon " described in Appendix A hereto (the "Project"); and

WHEREAS the Organization and DFO have a joint interest in the expected outcome of this collaboration and have shared or compatible objectives associated with the Project; and

WHEREAS the Organization and DFO are both expected to provide financial and/or in-kind resources towards the Project in accordance with their relative vested interest in the Project and understand that inkind resources provided for the Project shall be evaluated at cost; and

WHEREAS the Organization and DFO agree to a fair allocation of risk, demonstrated through the development of a governance framework on decision making, accountability, and risk mitigation related to the Project; and

WHEREAS this Agreement is neither a procurement agreement pursuant to the Government Contracts Regulations, nor a transfer payment agreement pursuant to the Treasury Board Transfer Payments Policy.

#### THEREFORE, the Parties agree as follows:

#### 1. Definitions

- a) "Agreement" means the recitals, definitions, terms, conditions and obligations stipulated herein including the stipulations in the appendices affixed hereto.
- b) "Contribution" means resources that are given/provided/supplied by a Party toward the Project. The term should not be confused with a Government of Canada Contribution, as per the Treasury Board Transfer Payments Policy.
- "Intellectual Property" or "IP" means any invention, and any other product of intellectual c) activity in the industrial, scientific, literary, or artistic fields including all intellectual creation

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legally protected through patents, copyright, industrial design, integrated circuit topography, and plant breeders' rights, or subject to protection under the law as trade secrets and confidential information.

- d) "Project Expenditures" means expenditures required for the Project, which are described and itemized in Appendix B of the Agreement.
- e) "Research IP" means IP arising from research and other activities performed under this Agreement, and any parts of such IP.
- f) "Crown" means the Federal Government of Canada.
- g) "Work Plan" means the detailed activities and corresponding resources required to implement the Project in accordance with the Project description provided in Appendix A.
- h) "Project Manager" means the person with authority to manage the Project and lead the planning and the development of all Project deliverables.

#### 2. Duration of the Agreement

a) The Agreement shall be effective as of the date of signature and shall expire, unless terminated sooner in accordance with article 15 of this Agreement on July 31<sup>st</sup>, 2012.

#### 3. Purpose of the Collaboration

The ACRDP directly supports DFO's Program for Sustainable Aquaculture, which reflects the federal government's commitment to increase scientific knowledge to support decision-making, strengthen measures to protect human health, and make the federal legislative and regulatory framework more responsive to the public and to the Aquaculture industry needs. The purpose and expected deliverables of the Project are described in Appendix A.

#### 4. DFO's Contribution

- a) DFO's contribution to the Project, estimated at \$76,758 represents the resources that DFO will provide to the Project as outlined in Appendix B.
- b) DFO contributes to the Project as follows:

Fiscal Year	Total value	List of DFO In-kind Contributions
2011-12	\$76,758	Staff time, travel, lab supplies

#### 5. Organization's Financial and/or In-kind Contribution

a) The Organization's contribution (financial and/or in-kind) to the Project, estimated at \$22,200 represents the resources that the Organization will provide to the Project as outlined in Appendix B.

b) The Organization contributes to the Project as follows:

Fisca Year		Total value	Financial Contribution	List of In-kind Contribution
2011-	12	\$22,200	\$6,000	Staff time, travel, lab supplies

c) The Organization shall make its financial contribution for the Project according to the payment schedule below:

Payment Amount	List of Deliverables	Estimated Date of Payment
\$6,000	Collections of tissues from jaundiced and healthy fish that can be used for transcriptional studies and pathogen isolation and sequencing in future Full functional genomics assessment of jaundiced fish, including the lists of genes and biological processes differentially regulated in response to the jaundice associated disease and an assessment of the potential roles of pathogens versus environmental perturbations in eliciting the disease.	June 15, 2011

- d) Amounts received by DFO under the Agreement will be deposited into a "Specified Purpose Account" ("Account") and used to pay for Project Expenditures. DFO shall not incur any Project Expenditures unless the Account contains enough funds to pay for such Project Expenditures. DFO will notify the Organization thirty (30) days in advance if it believes that funds remaining in the Account are not sufficient to cover anticipated Project Expenditures and the Organization shall provide funds in advance of the payment schedule herein.
- e) Project Expenditures for the supply of contracted goods and services to carry out the Agreement are subject to the Goods and Services Tax (GST) or the Harmonized Sales Tax (HST) as applicable.
- f) At the end of the Project or upon earlier termination of the Agreement, DFO will return to the Organization any money remaining in the Account after all monetary obligations in relation to the Project have been satisfied, unless the remaining amount is less than \$100 in which case the remaining funds will be credited to the CROWN as miscellaneous revenue.
- g) Throughout the Project and for two years after expiration or termination of the Agreement, the Organization may request access to DFO records related to Project Expenditures and DFO shall provide reasonable facilities and co-operation to allow the Organization to review these records and to take copies, as required.
- h) All payments shall be made payable to the Receiver General for Canada and delivered to DFO Finance:

Fisheries and Oceans Canada Revenue Unit Suite 200 – 401 Burrard Street Vancouver, BC V6C 3S4

#### 6. Risk Management

a) The Project Authorities as identified in section 7(b) of the Agreement have discussed and completed a risk assessment and analysis as per Appendix D of the Agreement.

#### 7. Project Authorities

- a) The Project shall be managed jointly, with each party separately administering its responsibilities under the Project. Each Party shall name a Project Authority to oversee the management and administration of its responsibilities with respect to the Project. The Project Authorities may call upon such other persons for assistance as they consider necessary.
- b) The Project Authority for DFO shall be:

Karia Kaukinen, MSc
Molecular Genetics,
Fisheries and Oceans Canada
Pacific Biological Station
3190 Hammond Bay Rd
Nanaimo, BC V9T 6N7
250 759-8358
Karia.Kaukinen@dfo-mpo.gc.ca

and the Project Authority for the Organization shall be:

Creative Salmon Company Ltd. PO Box 265 Tofino, BC VOR 2ZO 250-725-2884

c) Either Party may by notice request a change of address, or designate a new Project Authority.

#### 8. Monitoring and Evaluation

- a) The Parties shall ensure that the Project Authorities:
  - i) monitor the progress of the Project and performance of the Parties under the Annual Work Plan; and
  - ii) verify that Project Expenditures are consistent with anticipated Project costs and deliverables, as described in the Work Plan.

#### 9. Access to DFO Grounds and Buildings

a) The Organization, its employees and its agents involved in the Project shall abide by all orders and policies with respect to access to DFO sites, vessels and buildings and utilization of facilities therein, including orders and policies related to security, health and safety, and shall not bring any people, equipment or any materials into these buildings without the prior written consent of the DFO Project Manager.

#### 10. Reports and notices

- a) Each party shall report to the other on the progress of the research it is performing under the Project and any on scientific results and data arising from such research. Reports shall be provided at a reasonable frequency and at a minimum once annually to ensure that the Parties remain well informed and up-to-date on the Project. Each Party shall also provide to the other within Three (3) months from the termination or the end of the Project a final report on all the research it has performed under the Project, including the scientific results and data acquired from the research, and a summary of Project costs it has incurred. In addition, the Parties will cooperate to complete a final evaluation report for the entire Project within Three (3) months following the end of the Project, using the template provided in Appendix E, on the understanding that the DFO Project Authority will submit the report to the DFO ACRDP Regional Coordinator.
- b) If requested, the Organization shall assist the DFO Project Authority in preparing a fact sheet outlining the Project, the Project research methodology, and the Project results. It is agreed and understood that the final evaluation report may be used to develop the fact sheet, which DFO may publish on the DFO website as well as in hard-copy.
- c) Notices, reports and other communications under the Agreement shall be in writing and shall be addressed to the Project Authorities.

#### 11. Intellectual Property

- a) Each Party shall promptly disclose to the other any technology, data or other information in its possession, required by the other Party to perform any Project activities for which it is responsible. Each Party remains the owner of technology, data or information that it owned prior to disclosure to the other Party and such technology; data or information shall be subject to the confidentiality provisions of Appendix C.
- b) Research IP is subject to the provisions of Appendix C.

#### 12. Ownership of Biological Material and Equipment

- a) Any equipment and material, including animals, biological material and organisms arising, acquired, and produced under this Agreement belong to the Minister.
- b) Animals and biological material provided by the Organization for the Project and biological products issued therefrom, as defined in Appendix F of the Agreement shall be subject to the provisions of Appendix F.

#### 13. Dispute Resolution

a) If any dispute, other than a matter of public law arises between the Parties in connection with or arising out of the Agreement, the Parties shall use their best efforts to settle any such dispute by negotiations or mediation. If the Parties fail to resolve the dispute within a period of thirty (30) days or such greater period as may be mutually agreed, then either Party may refer the dispute to arbitration in accordance with the *Canadian Commercial Arbitration Act*. The location for the arbitration hearing shall be Nanaimo, BC.

#### 14. Liabilities

- a) Under no circumstances will Canada and its Ministers, officers, and employees incur any obligation or liability whatsoever for any death, injury, loss, damages, or expenses arising in any way, including gross negligence, in relation to the performance of the Agreement. Sole risk in this respect shall be borne by the Organization.
- b) The Organization shall indemnify and save harmless Canada and its Ministers, officers and employees from and against and be responsible for all claims, demands, losses, costs, damages, actions, suits or proceedings by whomever made, brought and prosecuted in any manner, based upon, arising out of, related to, occasioned by, or attributed to any acts or conduct of the Organization, its employees or agents relating to the Agreement unless such claims, suits, actions or demands result from injury, loss or damage caused by the negligence of a DFO employee acting within the scope of his employment

#### 15. Termination

- a) DFO may terminate the Agreement upon thirty (30) days notice to the Organization, if:
  - i) the Organization breaches any terms or conditions of the Agreement and does not remedy any such breach within thirty (30) days after being notified of the breach in writing by DFO; or
  - ii) The Organization is not conducting the Project in accordance with the Work Plan and does not rectify the matter within thirty (30) days after being notified in writing of the specific rectifications required to remedy the execution of the Project; or
  - iii) the Organization is insolvent, in receivership, bankrupt, files for bankruptcy, or is involved in any act of bankruptcy or any bankruptcy proceeding; or
  - iv) the Organization is convicted of any offense under any the law, order or regulation of Canada or the provinces or of a duly constituted authority thereof or the conditions of any license, or of being an accessory to any such offence, and if such offence is committed in connection with the Agreement; or
  - v) the Organization has submitted false or misleading information to DFO in respect of the Project or in respect of the Organization's obligations pursuant to the Agreement; or
  - vi) DFO is unable to continue supporting the Project due to departmental priorities and/or pressures; or

- b) DFO will amend or terminate the Agreement if the resources that DFO is expected to contribute to the Project (that are subject to corresponding appropriations being approved by Parliament) are reduced or not available. In either case, the Organization hereby agrees to refrain from taking any action against DFO, DFO Minister and DFO employees for damages.
- c) The Organization may terminate this Agreement by written notice to DFO if DFO is not conducting the Project in accordance with Appendix A and does not rectify the matter within thirty (30) days after being notified in writing of the specific rectifications required to the Project.
- d) If the Organization wishes to terminate the Agreement for any reason other than the one set out in paragraph (b), the Organization shall deliver to DFO a written request to terminate the Agreement and upon DFO receiving such a request DFO will deliver to the Organization a notice confirming any outstanding obligations, including financial obligations, under the Agreement and may terminate the Project and the Agreement if:
  - i) the Organization satisfies all its obligations and pays to DFO any amount required towards Project Expenditures that DFO is unable to suspend;
  - ii) ending the Project would not have a significant adverse effect on DFO; and
  - iii) the Organization is in full compliance with the terms of the Agreement.
- e) Expiration or termination of the Agreement shall not relieve the Organization from its obligations in respect of Intellectual Property, Publications and Confidentiality as set out in Appendix C to this Agreement.
- f) Failure by DFO to notify the Organization of a breach of the Agreement or to terminate the Agreement because of such breach shall not constitute an acceptance of the breach or a waiver of the right of DFO to terminate this Agreement in accordance with its provisions, and to recover any sums due to DFO under the Agreement.

#### 16. Canadian Environmental Assessment Act (CEAA)

a) The Organization and DFO shall ensure that, if applicable, the Project is assessed and approved in accordance with the Canadian Environmental Assessment Act prior to commencing the Project.

#### 17. Canadian Council on Animal Care (CCAC)

a) The Organization and DFO shall ensure that, if applicable, the Project is assessed and approved in accordance with the standards of the Canadian Council on Animal Care (CCAC). DFO will contact its Animal Care Committee to ensure compliance with this provision prior to commencing the Project.

#### 18. General

#### a) Entire Agreement

The Agreement, which includes the Appendices appended thereto and which are part thereof, sets

forth the entire agreement between the Parties hereto concerning the subject matter hereof and supersedes and revokes all negotiations, arrangements or communications, of any nature whatsoever whether they be verbal or in writing, between the Parties or their authorized representatives or any other person purporting to represent DFO or the Organization.

#### b) No Agency

Nothing contained in the Agreement shall be considered or construed a creating the relationship of partners, principal and agent, lessor and lessee, licensor and licensee (except with respect to Research IP, in accordance with Appendix C to the Agreement) or of employer and employee between the Parties. In particular, the Organization agrees to be solely responsible for any and all payments and/or deductions required to be made including those required for Canada Pension Plan, Employment Insurance, Workers' Compensation, or Income Tax for all its employees involved in the Project. The Organization shall be solely responsible for the supervision, scheduling of work and tasking for its employees and agents engaged by or on behalf of the Organization for the Project.

#### c) House of Commons

No member of the House of Commons shall be admitted to any share or part of the Agreement or to any benefit that may arise from it.

#### d) Public Servants

No former public office holder who is not in compliance with the post employment provisions of the Values and Ethics Code for the Public Service shall derive a direct benefit from the Agreement.

#### e) Laws in force

The Agreement shall be interpreted in accordance with federal laws of Canada and the laws in force in the Province of British Columbia.

#### f) Location

The Project shall be performed at Pacific Biological Station 3190 Hammond Bay Rd , Nanaimo , in the Province of British Columbia.

#### g) Amendment

This agreement may only be amended by a written amendment signed by the Parties' authorized representative at any time during the term of this Agreement.

#### h) Severability

Should a court of competent jurisdiction hold that any provision of the Agreement is invalid, illegal, or unenforceable, such provision shall be considered severed from the Agreement and all other provisions of the Agreement, and all rights and obligations therein shall continue to be in force and effect.

#### i) No Assignment

Neither Party may assign the Agreement, in whole or in part, without the prior written consent of the other Party(s).

#### j) Official Languages

- i) The Agreement was prepared in English at the request of the organization; and
- ii) All announcements and communications to the public concerning the Project or this collaboration shall be made in both official languages.

## k) Lobbyist Registration Act

Any person lobbying on behalf of the Organization must be registered pursuant to the Lobbyist Registration Act.

#### 1) Time of Essence

Time is of the essence with respect to all deliverables under the Agreement.

#### m) Order of Precedence

If there is a conflict of ambiguity between this Agreement proper and any appendices or schedules thereto, this Agreement proper shall prevail.

IN WITNESS WHEREOF the Agreement has been executed by DFO and the Organization through their duly authorized officers.

The Organization:	Her Majesty the Queen in Right of Canada, as represented by the Minister of Fisheries and Oceans.
	LRichards
Signature	Signature JUN - 8 2011
Name & Title of Organization's authorized representative	Dr. Laura J. Richards Regional Director Science
Date	Date
i/ui/	Date

#### Appendix A: Project Description / Work Plan

Observed with unique clinical presentation, the salmon present with mild to severe yellow discolouration of the skin (jaundice). This is most evident on the abdomen and around the eyes. These fish also have very pale gills indicating anemia. Internal signs include pale livers and often the stomachs of the fish are empty indicating the fish have not eaten for a number of days although the overall condition of the fish is good. Grossly the other organs appear unaffected.

The clinical presentation is very different from Marine Anemia syndrome, another Chinook salmon disease, which typically presents with splenomegaly, renomegaly, and anemia.

Histological examination has found severe liver and kidney damage (hydropic degeneration). The proposed etiology includes a pathogen or exposure to a negative environmental influence (hereafter referred to as an undefined toxin). Repeated testing using traditional diagnostic tests have been unable to identify a pathogen. Tests including classical bacterial culture, viral cell culture, PCR, blood assessment and histopathology have yielded negative results for pathogens including Renibacterium salmoninarum (BKD), Listinella sp (vibriosis), VHSV, IHNV, ISAV, VEN, EIBS, Loma salmonae, and Nucleospora salmonis (marine anemia).

Little is known of the epidemiology of the condition. It affects fish that have been in sea water for greater than 6 months and therefore is not considered related to smolt quality. There appears to be a seasonal pattern to this condition with clinical signs and mortalities observed late fall/early winter (December) spiking in the winter and apparently resolving by early summer.

This condition is most typically observed at the farm site operated by Creative Salmon that contains the greatest freshwater influence, which is one reason to suspect an environmental effect may be at play, although it is sometimes observed at relatively lower incidence levels at other farm sites. It has been seen in most of the generations stocked at the freshwater-influenced farm even though the company operates single year class sites with a fallow period before re-stocking. At the most affected farm most often one or two pens of fish are severely affected, however the condition may be seen in many of the pens. The mortalities levels in most heavily affected pens typically would be several folds higher than the other pens on the farm. For example in January 2011, the single affected pen (of an 8 pen site) disproportionately made up 35% of the mortalities grossly examined. Of the mortalities examined from this single pen over 77% of the fish examined exhibited jaundice. Total mortality attributed to this condition has not been fully assessed although at certain times of the year it can be as significant as other diseases.

Currently there are no tools available to manage the problem. A better understanding of the epidemiology and etiology would enable us to develop these tools.

#### Project objectives

Our main objective is to apply functional genomics technology to gain a better understanding of the factors that may underlie the poorly understood jaundice-related disease experienced by farmed Chinook salmon in Tofino. Of most import, Creative Salmon managers need to know whether this disease is more likely the result of an infectious agent or environmental conditions.

If the genomic signature indicates that an infective agent is likely involved, they will pursue the identification of this agent through 454 sequencing of affected tissues in a follow up study. If the

genomic signature is more likely associated with environmental conditions at this farm site, e.g. low salinity, toxicants, or other factors, follow-up studies could be performed to assess the most likely environmental mechanisms. In either case, knowledge of the mechanisms leading to this disease will potentially provide managers with tools to track, predict, and/or potentially mitigate the impact of this disease in future.

Our other objective is to improve our understanding of the epidemiology of the condition — why is the condition more prevalent at one farm as compared to the others in the same area, or in some pens and not others? This would include examining the mortality pattern, and determining overall mortality attributed the condition, and the environmental factors associated with it.

#### Description of work and experimental protocol

Fish on Creative Salmon's farms are regularly screened for health, and the presence of jaundice is one of the metrics that is tracked bi-weekly in each net pen at each site. While mild jaundice can occur to some degree at all sites, jaundice-related mortality is predominant at the farm with the greatest freshwater influence. Despite extensive histological study, we still do not understand whether the jaundice may result from an infective agent or may be a manifestation of environmental conditions, possibly relating to low salinities, in the winter, or a combination of both. Hence, we need to design an experiment that considers both possibilities.

The molecular genetics laboratory has successfully used microarray approaches on wild fish to assess unknown factors associated with poor performance (e.g. Miller et al. 2010) and transcriptional responses to shifting environments (Miller et al. 2009; Evans et al. In Review).

We have also conducted microarray studies of host response to disease (Miller et al. 2007; IHNV) and have conducted studies with Peter Ross at IOS on the influence of toxicant exposure on immune response to Vibrio (data still in preparation for publication). Moreover, we have been working closely with a bioinformatics group at UBC, lead by Dr. Paul Pavlidis, on the development of meta-analysis tools to identify correlated profiles among microarray experiments. Hence, we have the experience and expertise to undertake this exploratory study.

We will use similar experimental designs and approaches as we have used in previous studies (e.g. Miller et al. 2009) whereby we apply balanced replication across each biological variable, or treatment, of interest. Here, treatments are not really treatments, as one would define in a controlled laboratory study, but rather biologically meaningful entities, like sample sites, disease state, and life-history stage.

Approach: If an infective agent is involved, it is important to determine whether it is present in smolts used to stock the farm sites, or whether it likely emanates from the marine environment. As such, our study will include smolts used to stock net pens in 2011 (Treatment 1; note that the sample size of smolts would only detect positives that affected 10% or more of fish, so it is not an exhaustive, definitive assessment of the role of freshwater). To control for the environment, we will contrast apparently healthy (no evidence of jaundice) fish a farm that is not substantively affected by jaundice (Treatment 2) with healthiest fish that can be obtained sampled from the affected freshwater-influenced farm (Treatment 3). To obtain healthy fish, we will chum fish from netpens that have been shown to have the lowest levels of jaundice and conduct histological analyses to ascertain their state of health. Fish that were collected in this manner but deemed to be positive for,

but not dying of, jaundice at the time of collection will also be included in the analysis to control for the effects of morbidity (Treatment 4). Finally, moribund fish with clear evidence of jaundice will be included in the study (Treatment 5). Multiple tissues from all collected fish will be examined histologically.

Head and Posterior kidney, spleen, liver, heart, muscle, gill, and blood of 10-15 fish will be collected for each "treatment" category.

I. Smolts

II. Healthy fish from unaffected farm at mouth of the inlet

III. Healthy fish from affected farm

IV. Fish with histological signature of jaundice but not moribund at affected farm

V. Moribund fish with histological signature of jaundice at affected farm In anticipation of this study, some of the samples of moribund and jaundiced fish were collected during a mortality event at the end of February 2011. Additional samples of jaundiced but not moribund and healthy fish from both farm sites will be collected in early March, along with additional moribund and jaundiced fish, if still present. Smolts will be collected in April before they are put to sea.

The microarray study will be performed on cDNA from liver tissue, as liver is one of the most severely affected organs and is also the primary tissue for detoxification. We will keep the remaining tissues for potential future study. In a balanced experimental design, twelve biological replicates (individuals) will be included for each of the five treatments, with the total study comprising 60 arrays. The Salmonid Agilent 4x44K olignucleotide arrays developed through the cGRASP program by Ben Koop's laboratory at University of Victoria will be used in the study, with approaches similar to those we have used in other studies (e.g. Miller et al 2009, 2011). A reference sample comprised of liver cDNA from all of the individuals used in the study (labelled with Alexa555) will be hybridized along with the experimental sample (labelled with Alexa647) on each array. After slide quality assessment and Lowess normalization, arrays will be statistically analysed using both supervised (multifactorial ANOVA with posthoc testing via Mann Whitney U t-test) and unsupervised (Principle Component Analysis) approaches to identify transcriptional differences among treatments and the main physiological trajectories in the data. Functional analysis of the biological processes over-represented among the differentially regulated genes will be determined using the programs DAVID (http://niaid.abcc.ncifcrf.gov/) and PathwayStudio (Ariadne Genomics). Further information on the functional role of the most significantly differentially regulated genes will be gleaned from the protein literature mining website ihop (http://www.ihop-net.org/UniPub/iHOP/). Many salmon diseases have already been characterized by microarrays (e.g. Miller et al. 2007, Rise et al. 2004, Morrison et al. 2006, Baerwald et al. 2008), including the infectious diseases ISAV, IHNV, VHSV, Aeromonas salmonicida, Amoebic gill disease, salmon louse, and others. A wealth of microarray studies also exist for toxicant responses in fish (e.g. Finn et al. 2007, Tilton et al. 2006, Hook et al. 2006) for a diverse array of chemicals, including PBDE (flame retardant), endocrine disrupting compounds, heavy metals, PCB's, and others. In our own research, we also have data from both wild fish and controlled laboratory studies that assesses responses to shifting salinities and temperature, and we have assessed wild salmon in the ocean at the same time of the year as the fish from this study (the first winter at sea), some even from the west coast. We will use these studies as a backdrop to assess the possible correlation of signatures emanating from

jaundiced Chinook salmon with signatures associated with pathogenic disease, toxicant exposure and other environmental stressors. To do this, we will use the recently develop meta-analysis program for microarray data GEMMA (http://www.chibi.ubc.ca/maintenance.html), developed from our colleague and collaborator on our wild salmon studies, Paul Pavlidis. The literature mining software available within PathwayStudio will be similarly applied.

Because the farm site most affected by the jaundice-related disease also contains the lowest salinities of all the farm sites, we will additionally address the potential role that osmoregulatory dysfunction may play in the manifestation of this disease. We will assess the osmoregulatory state of fish in each treatment through quantitative PCR of gill cDNA for the isoforms of Na+ K+-ATPase, cold-inducible RNA binding protein, prolactin, and growth hormone. We will also determine osmolality and ion concentrations in blood plasma, which will indicate whether fish are able to maintain homeostasis in their gills.

We will evaluate health records collected and environmental data collected for the last seven years for the farm where the jaundice is most prevalent. Similar data will also be collected from one other farm where jaundice has not been observed or has been observed at very low prevalence. Health data is collected twice a week over the duration that the fish are at sea (~18-20mos). Environmental data (temperature, salinity and dissolved oxygen) is collected on a daily basis. The data will encompass 3 different generations stocked on the farm. These records will be used to estimate prevalence and describe the pattern of the disease both temporally (i.e. time of onset, age of onset, duration of disease, environmental profile) and spatially (difference in prevalence between years, between pens, between farm sites). This will be the first epidemiological analysis of this condition.

#### Detailed deliverables

Collections of tissues from jaundiced and healthy fish that can be used for transcriptional studies and pathogen isolation and sequencing in future.

II. Full functional genomics assessment of jaundiced fish, including the lists of genes and biological processes differentially regulated in response to the jaundice associated disease and an assessment of the potential roles of pathogens versus environmental perturbations in eliciting the disease.

III. A list of genes that might be useful biomarkers to predict disease and stage disease progression

IV. Characterization of osmoregulatory state of fish at the two farm-sites through biomarker screening of gill tissue and plasma ion and osmolality levels

V. Epidemiological analysis of the disease prevalence at each of the farm sites over the past 7 years and environmental data

VI. Recommendations to industry on next step research to either identify an infective agent associated with the disease or to narrow down potential environmental factors involved.

VII. Manuscript to be published in a peer-reviewed journal describing results (will not be complete until after the project ends)

# **Appendix B: Project Expenditures**

# Budget Summary by Fiscal Year 2011-12 (1 April – 31 March)

	Organization		Department of Fisheries and Oceans			
Description	Financial Contribution	In-Kind Contribution	ACRDP Contribution	Other DFO In-Kind Contribution		
Salary						
. Miller IND RES -03		<u> </u>		4,000		
Kaukinen IND BI-02						
CAHS Veterinarian-						
Equipment						
Material & Supplies	6,000					
Travel						
Facilities						
Grand Total	6,000	16,200	72,758	4,000		

# Salaries

Salary for ½ year of a BI-02 (Karia Kaukinen) in the molecular genetics laboratory who will be responsible for
project management and reporting, RNA extractions, biomarker qPCR, microarray experiment, and preliminary data
analysis provided by ACRDP. Karia's salary per year. ( *1.2 benefits = , funded
from industry, from ACRDP
Dr. Miller will dedicate of her time to the project, at an in kind cost of \$4,000. Most of this time will be spent on
data analysis and interpretation

#### Appendix C: Intellectual Property, Confidentiality and Publication

#### 1. Rights with respect to Research IP

- 1.1. Research IP that is created, developed or produced by DFO employees in the course of their employment, or with any intellectual contribution or direction from DFO employees shall belong to Canada, under the control and administration of the Minister. Research IP that is created, developed or produced by the other Party without any contribution or direction from DFO employees shall belong to the other Party.
- 1.2. If a Party creates, develops, or produces any Research IP such Party shall promptly disclose such Research IP to the other Party and provide to the other Party all technical information that may be necessary to enable the other Party to use the Research IP. The other Party may use the Research IP for non-commercial research purposes only, without restrictions and without any obligations to the first Party, but may not use it in any other way or disclose it to third parties without the prior written authorization from the first Party.
- 1.3. The Party collaborating with the Minister under this Agreement may request a licence from the Minister to use Canada-owned Research IP for commercial exploitation. The request shall be in writing, and delivered to the Minister no later than Three (3) months after the end of the Project. The Parties agree to negotiate in good faith the terms and conditions of a licence, however if they can't agree on the terms and conditions within Three (3) months following the beginning of licence negotiations, or at such later time as the Parties may agree, the Minister will no longer be obligated to continue negotiating a licence with the other Party.

#### 2. Patenting of Research IP

- 2.1. The Parties shall fully cooperate with each other, and assist each other free of charge in the preparation and filing of any patent applications related to Research IP, including without limitation obtaining the necessary assistance to prepare patent applications.
- 2.2. Each Party shall promptly provide to the other a copy of every patent application that it files in relation to Research IP.
- 2.3. Each Party shall execute such conveyances or other documents as required for the filing, prosecution and maintenance of any patent applications, and for defending any issued patents related to Research IP.

#### 3. Confidentiality

3.1. Any technology data or other information of any kind related to the Project (Collectively "Information") shall be deemed confidential and neither Party may release any such information to others in any way whatsoever without the prior written authorization of the other Party. However this confidentiality obligation shall not apply to the Party who owns the Intellectual Property in such Information, and in the case of DFO, this confidentiality obligation shall be subject to the access to information and privacy protection legislation, including the Access to Information Act and the Privacy Act.

3.2. A Party who receives confidential Information transmitted in any form by the other Party shall keep that Information confidential. However this obligations shall not apply to Information, that is or falls lawfully in the public domain, that was lawfully in the possession of the Party prior to disclosure by the other Party, or that a Party may receive from a third party not bound by any confidentiality obligations.

#### 4. Publication and Disclosure of Information

4.1. If a Party ("First Party") wants to disclose any Information produced under this Agreement, other than Research IP owned by the First Party, it shall submit the information intended for disclosure to the other Party for review, at least sixty (60) days prior to the intended disclosure. The other Party will have thirty (30) days to notify the First Party in writing if such information or any portions thereof must be withheld from disclosure for patenting purposes, or for the purpose of a scientific publication. Upon being so notified the First Party may either delete from the intended disclosure the information that the other Party has requested be withheld from disclosure or withhold disclosure of such information for a reasonable time to allow the publication or the filing of a patent application. Any request to withhold disclosure for may extend for a reasonable time but not exceeding one year.

#### 5. Term of obligation

5.1. The obligations of the Parties herein shall survive the expiration or termination of the Agreement to which this Appendix is affixed and of which it is part. However in respect of confidential information any confidentiality obligation shall remain in effect until such time that the information becomes public.

# Appendix D: Risk Management

It is the government policy to identify and reduce or eliminate risks, minimize and contain the costs and consequences of harmful or damaging incidents arising from these risks.

http://www.tbs-sci.gc.ca/pubs\_pol/dcgpubs/RiskManagement/riskmanagpol\_e.asp\_http://www.tbs-sci.gc.ca/pubs\_pol/dcgpubs/RiskManagement/guide\_e.asp\_

			Project	Project Risk Analysis	alysis		
Project Objective	Anticipated Risk Description and its	Result of likelihood a impact assessment	tesult of likelihood and impact assessment	Risk		Additional mitigation action	Manager Responsible
	Consednences	Likelihood	Impact	Kating	capacity or capability	or strategy	(see governance framework)
To apply functional Those mortality	Those mortality	Minimal	moderate	3	We have initial outbreak	None required	Karia Kaukinen
genomics technology events may have	events may have				samples of moribund fish		
to gain a better	been already over				from February 2011, but we		
understanding of the prior to the	prior to the				plan to augment this small		
factors that may	commencement of				sample set with larger		
underlie the poorly   the project and	the project and				sample sets that include		
understood jaundice- samples may not be	samples may not be				negative and positive		
related disease	available.				controls.		
experienced by					,		
farmed Chinook							
salmon in Tofino.							

# Appendix E

# **ACRDP Final Project Report**

## PART I

- 1. Project #:
- 2. Project Title:
- 3. Project Duration:
- 4. Project Leader, contact information:
- 5. Industry partner(s):
- 6. Expenditures and variance from budget:

	Contribution	Initial budget	Actual expenditure	Difference
Industry \$				
Industry (in kind)				
ACRDP (\$)				
Other DFO (\$ and in kind)				
Partners (\$ and in-kind)				

- 7. Expertise developed during the project (e.g., within DFO, industry, graduate students etc.):
- 8. General Comments:

#### PART II

- The following sections should be completed in a non-technical language that is suitable for an audience comprising individuals involved in the aquaculture industry.
- Please limit the information to approximately 5 pages
- 9. Project rationale (e.g., background information, why solving the problem was of interest to industry, project hypothesis and goals):
- 10. Short summary of project methods (e.g., experimental and analytical procedures followed, deviations from the originally proposed methods):
- 11. Key results (include graphs, data tables, photos, etc. where applicable):
- 12. Resulting key improvements to sustainable aquaculture and scientific advancements:
- 13. Suggested next steps, future research/development/innovation needs:
- 14. Copies of publications, reports or articles produced in reference to the project:
- 15. Identify any invention or innovation that may have resulted from this Project, including any new process or technique.

# PART III

Declaration:		
Iknowledge the report is accu	have completed the report and declare that to the best of arate.	my
Signature	Date	
Approved by:		
DFO Project Authority	Date	
Industry Project Authority	 Date	

## Appendix F

#### **Provisions related to Biological Products**

#### **Definitions**

- 1. "Biological Products" means animals, biological material and organisms.
- 2. "Biological Material" means Biological Products provided by the Organization to the Minister for the Project.
- 3. "Issued Biological Material" means Biological Products issued from Biological Material, and otherwise acquired, and produced under the Project.

#### Biological Material belongs to the Organization, and Issued Biological Material belongs to DFO

- 1. Issued Biological Material shall belong to the Minister.
- 2. Ownership of Biological Material shall remain with the Organization, and the Minister shall return such Biological Material to the Organization at the end of the Project; it is agreed and understood that the Minister shall not be responsible for the condition of any Biological Material, or for death of any animals in its possession, and will not return any deceased animals to the Organization.
- 3. The Organization shall not dispose of, or transfer any Biological Products returned by the Minister to the Organization without the Minister's prior written authorization, such authorization not to be unreasonably withheld. It is agreed and understood that such authorization may be withheld if the proposed disposal and transfer of the Biological Products might present an environmental risk or might jeopardize the Minister's rights and interests in any Intellectual Property relating to the Biological Products.

#### Parsons, Jay

From:

Parsons, Jay

Sent:

Friday, November 24, 2017 3:24 PM

To: Cc:

Moore, Wayne

Subject:

White, Andrea RE: Summaries - 2 papers

MECTS-#3852940-v1-2017

Attachments:

\_EOS\_SRS\_ABAAHS\_Jaundice\_in\_Chinook\_Salmon\_on\_the\_west\_coast\_of\_British\_Columbi

a.DOCX

Importance:

High

Wayne,

For your review and approval.

Jay

From: Moore, Wayne

Sent: Thursday, November 23, 2017 8:06 PM

To: McPherson, Arran; Parsons, Jay

Cc: White, Andrea

Subject: Re: Summaries - 2 papers

Of course. We live to serve :-).

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: McPherson, Arran

Sent: Thursday, November 23, 2017 7:11 PM

To: Moore, Wayne; Parsons, Jay

Cc: White, Andrea

Subject: Summaries - 2 papers

Hi Wayne and Jay, is it possible to provide a plain language overview of the findings of the 2 papers by cob tomorrow. Also, are we planning any further work in this area. Thanks, Arran.

# Pages 1661 to / à 1662 are withheld pursuant to sections sont retenues en vertu des articles

14(a), 21(1)(b), 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

#### Jaundice in Chinook Salmon on the West Coast of British Columbia

A Jaundice Syndrome occurs sporadically among sea-pen-farmed Chinook Salmon grown on the west coast of Vancouver Island, British Columbia. Affected salmon are easily identified by a distinctive yellow discolouration of their skin around their abdomen and eyes. This syndrome can result in mortalities to the fish, generally in the fall-winter periods. Little is known about the cause, incidence or distribution of this disease, including whether it is infectious or non-infectious.

In 2011, a collaborative project, funded through the DFO Aquaculture Collaborative Research and Development Program (ACRDP) was initiated among Creative Salmon Ltd, DFO (Pacific Biological Station) and other research partners. The study was design to gain a better understanding of the factors that might be causing jaundice in the Chinook Salmon; whether they are related to the environmental conditions or an infectious agent. A fish health diagnostic approach (including histopathology) and a genomics approach were used to investigate the cause(s).

The project was completed in 2012 and a draft report was produced<sup>1</sup>, but not finalised as there was and still is a fundamental disagreement among the researchers involved on the project regarding the final conclusions of the study. While the study did find that environmental factors are implicated in the level and occurrence of jaundice in the fish due to lower salinity conditions, part of the research team contends that there is a direct and conclusive link between jaundice in Chinook Salmon and a virus (Piscine Reovirus - PRV), while other researchers that were part of the team contend that while there may be a possible link to PRV, based on the findings of this study there is not a conclusive connection to PRV and that it is not the only factor involved in causing the disease.

<sup>1</sup> ACRDP project draft manuscript: Histopathology and genomic characterization of idiopathic jaundice and anemia syndrome in cultured Chinook salmon (*Oncorhynchus tshawytscha*) by Kristina M. Miller, Karia H. Kaukinen, Shaorong Li, Angela Schulze,

Gary D. Marty, and

## Molecular indices of viral disease development in wild migrating salmon

In 2017, researchers at the DFO Pacific Biological Station published a study on molecular indices of viral disease in salmon (Molecular indices of viral disease development in wild migrating salmon by Kristina M. Miller, Oliver P. Günther, Shaorong Li, Karia H. Kaukinen and Tobi J. Ming in Conservation Physiology (2017) vol. 5(1): cox036; doi:10.1093/conphys/cox036). This study examined a series of molecular biomarkers

(measureable biological molecules that are characteristic for a specific physiological status) to determine the disease state of a fish. This new genomics-based approach offers a new and different, more sensitive approach compared to traditional fish health diagnostic approaches to determining the disease state of a fish. The technique has the potential to be able to determine if a fish is in an active infectious state even before a disease state may be reached, which can be a particular challenge to assess in wild fish stocks.

This study developed and validated salmon host biomarkers capable of distinguishing fish in an active viral disease state from those carrying a latent viral infection, and viral versus bacterial disease states. Biomarker discovery was conducted through a complex statistical analysis of published and in-house genomic data, and validation performed on independent datasets including disease challenge studies and farmed salmon diagnosed with various viral, bacterial and parasitic diseases. One of these datasets included the analysis of fish and findings from the ACRDP study on jaundice in farmed Chinook Salmon (see above). The specific finding associated with jaundice included the identification of some biomarkers that were able to distinguished between healthy and diseased Chinook, as well as noting a correlation with PRV.

#### Other Research

Other research on jaundice has been recently undertaken by research at the DFO Pacific Biological Station and includes a study examining jaundice and PRV on salmon (Piscine reovirus, but not Jaundice Syndrome, was transmissible to Chinook Salmon, *Oncorhynchus tshawytscha* (Walbaum), Sockeye Salmon, *Oncorhynchus nerka* (Walbaum), and Atlantic Salmon, *Salmo salar* L. by K A Garver, G D Marty, S N Cockburn, J Richard, L M Hawley, A Muller, R L Thompson, M K Purcell and S Saksida in the Journal of Fish Diseases (2015) doi:10.1111/jfd.12329.

The results from this study demonstrate that the Jaundice Syndrome was not transmissible by injection of material from infected fish and that PRV was not the sole causative factor for the condition. Additionally, these findings showed the Pacific coast strain of PRV, while transmissible, was of low pathogenicity for Atlantic Salmon, Chinook Salmon and Sockeye Salmon.

From:
-------

Miller-Saunders, Kristi

Sent:

November-26-17 7:37 PM

To:

Brian Riddell

Subject:

FW: Summary information needed for PRv-HSMI Workshop

Attachments:

PRV-HSMI-current information-DRAFT.pptx

FYI

**From:** Marty, Gary D AGRI:EX [Gary.Marty@gov.bc.ca]

Sent: November 26, 2017 1:21 PM

To: 'Maureen Purcell (mpurcell@usgs.gov)'; Miller-Saunders, Kristi; 'Kathleen Frisch'; Garver, Kyle; Johnson, Stewart;

Gagne, Nellie; 'Tony Farrell (tony.farrell@ubc.ca)'; DiCicco, Emiliano; Polinski, Mark; 'Espen Rimstad (espen.rimstad@nmbu.no)'; 'George Iwama

Cc:

Subject: Summary information needed for PRv-HSMI Workshop

Workshop Title: Exploring PRv & HSMI in Europe & BC

Dear Presenters,

I am looking forward to hearing your presentation at the workshop.

For the final session of the workshop (Tuesday afternoon), "Regional comparisons & next steps: Are we on the same page & where do we go from here? – Structured Speaker Panel Discussion", I seek your assistance:

1. I am preparing to introduce the session with a brief summary of PRV and HSMI in Europe and BC (draft attached). Some of my draft entries might be incorrect or incomplete.

**REQUEST** - Please send me any additions, corrections, or other suggestions for the attached file.

2. I am also planning to present a summary of the most important information from each presentation. [I want to be sure to include information from talks that do not directly address PRV or HSMI in Europe or BC (but still provide important perspective).]

**REQUEST** - Please send me one to three bullets that summarize the "take home" message(s) from your presentation.

I will be able to integrate written notes into my summary if I receive them before 10pm Monday evening. Otherwise, I will do my best to integrate what I hear from you directly or during your talk (I will be taking notes during the sessions).

Best regards,

Gary

Gary D. Marty, Senior Fish Pathologist Animal Health Centre Ministry of Agriculture 1767 Angus Campbell Rd. Abbotsford, BC, V3G 2M3 604-556-3123

s.19(1)

# PRV – current information

PR	PRV – current information	formation (months)
	Norway	) BC
First Detection	(Palacios et al. 2010)	Sept. 13, 2010 (BC Animal Health Centre, unpublished) 2012 (Kibenge et al. 2013)
Earliest detection	<b>c</b>	1987 – several PCR+ results in wild and farm salmon (Marty et al. 2015)
Marine farm salmon prevalence	Most salmon farms are PCR+	The majority of farm salmon are PCR+ after 4 - 6 months at sea
Marine wild salmon prevalence	13% (Atlantic salmon, Garseth et al. 2014)	0 – 20% (Miller et al. 2014; Marty et al. 2015)
Causes subclinical HSMI?	Yes (Wessel et al. 2017)	No (Garver et al. 2016a)
Affects stress test performance?	<b>~</b>	To be presented(?) (Farrell et al. unpublished)
Causes clinical HSMI?	No (Wessel et al. 2017)	No (Garver et al. 2016b)
Occurs with HSMI?	Yes (Palacios et al. 2010)	Yes (Di Cicco et al. 2017)

# **HSMI** – current information

Earliest re idiopathic cardiomyc cardiomyc cardiomyc cardiomyc cardiomyc of consiste and skelet inflammat inflammat on affecte on affecte Marine wi salmon presalmon p	Earliest report of ? November 1990 (Brackett et al. 1991)	First public report 1999 (reported in Kongtorp 2013 (GD Marty. 2013; Canadian of consistent heart et al. 2004)  and skeletal muscle inflammation ONE)	Estimated mortality $0-20\%$ (Kongtorp et al. ~0.2% (source: Di Cicco et al. 2017. on affected farm(s) 2004)	Marine wild 0% 0% salmon prevalence (n = 21 PRV+ fish; Garseth (n = 204; Marty et al. 2015) et al. 2014)	Subclinical disease Yes To be presented(?) (Kongtorp et al. 2004)	Clinical disease No No (Camissible)
--	---	---	--	--	---	-------------------------------------

### 001668

## Literature Cited

Brackett, J., G. Newbound, and D. Speare. 1991. A fall survey of saltwater morbidity and mortality in farmed salmon in British Columbia. British Columbia Ministry of Agriculture and Fisheries.

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orthoreovirus from western North America is transmissible to Atlantic salmon and sockeye salmon but fails to cause heart and skeletal Garver, K.A., S.C. Johnson, M.P. Polinski, J.C. Bradshaw, G.D. Marty, H.N. Snyman, D.B. Morrison, and J. Richard. 2016a. Piscine muscle inflammation. Plos One 11(1) Garver, K.A., G.D. Marty, S.N. Cockburn, J. Richard, L.M. Hawley, A. Muller, R.L. Thompson, M.K. Purcell, and S. Saksida. 2016b. Piscine reovirus, but not jaundice syndrome, was transmissible to Chinook Salmon, Oncorhynchus tshawytscha (Walbaum), sockeye salmon, Oncorhynchus nerka (Walbaum), and Atlantic Salmon, Salmo salar L. J. Fish Dis. 39(2):117-128.

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### 001669

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From:

Brian Riddell <bri>ddell@PSF.CA>

Sent:

November-26-17 9:24 PM

To:

Miller-Saunders, Kristi

Subject:

Re: Summary information needed for PRv-HSMI Workshop

Thanks, not sure my week will be much better! Glad you and Emiliano are going, thanks.

On Nov 26, 2017, at 9:16 PM, Miller-Saunders, Kristi < <a href="mailto:Kristi.Saunders@dfo-mpo.gc.ca">Kristi.Saunders@dfo-mpo.gc.ca</a> wrote:

Not particularly looking forward to this meeting, but Emiliano has done a fantastic job on the in situ's. I will forward you a link to our talk on dropbox in the morning.

Kristi

From: Brian Riddell [briddell@PSF.CA<mailto:briddell@PSF.CA>]

Sent: November 26, 2017 8:54 PM

To: Miller-Saunders, Kristi

Subject: Re: Summary information needed for PRv-HSMI Workshop

On Nov 26, 2017, at 7:36 PM, Miller-Saunders, Kristi < <a href="mailto:Kristi.Saunders@dfo-mpo.gc.ca">Kristi.Saunders@dfo-mpo.gc.ca</a> wrote:

FYI

From: Marty, Gary D AGRI:EX [Gary.Marty@gov.bc.ca<mailto:Gary.Marty@gov.bc.ca><mailto:Gary.Marty@gov.bc.ca>]

Sent: November 26, 2017 1:21 PM

To: 'Maureen Purcell (mpurcell@usgs.gov<mailto:mpurcell@usgs.gov><mailto:mpurcell@usgs.gov>)'; Miller-Saunders, Kristi; 'Kathleen Frisch'; Garver, Kyle; Johnson, Stewart; Gagne, Nellie; 'Tony Farrell

 $(\underline{tony.farrell@ubc.ca}{\times}mailto:tony.farrell@ubc.ca}{\times}mailto:tony.farrell@ubc.ca})'; DiCicco, Emiliano; Polinski, Mark; Conv. Co$ 

'Espen Rimstad (espen.rimstad@nmbu.no<mailto:espen.rimstad@nmbu.no><mailto:espen.rimstad@nmbu.no>)';

'George

Iwama (

s.19(1)

s.21(1)(a)

s.21(1)(b)

Con	
LL.	

Subject: Summary information needed for PRv-HSMI Workshop

Workshop Title: Exploring PRv & HSMI in Europe & BC

Dear Presenters,

I am looking forward to hearing your presentation at the workshop.

For the final session of the workshop (Tuesday afternoon), "Regional comparisons & next steps: Are we on the same page & where do we go from here? - Structured Speaker Panel Discussion", I seek your assistance:

1. I am preparing to introduce the session with a brief summary of PRV and HSMI in Europe and BC (draft attached). Some of my draft entries might be incorrect or incomplete.

REQUEST - Please send me any additions, corrections, or other suggestions for the attached file.

2. I am also planning to present a summary of the most important information from each presentation. [I want to be sure to include information from talks that do not directly address PRV or HSMI in Europe or BC (but still provide important perspective).]

REQUEST - Please send me one to three bullets that summarize the "take home" message(s) from your presentation.

I will be able to integrate written notes into my summary if I receive them before 10pm Monday evening. Otherwise, I will do my best to integrate what I hear from you directly or during your talk (I will be taking notes during the sessions).

Best regards,

### Gary

Gary D. Marty, Senior Fish Pathologist Animal Health Centre Ministry of Agriculture 1767 Angus Campbell Rd. Abbotsford, BC, V3G 2M3 604-556-3123

<PRV-HSMI-current information-DRAFT.pptx>

s.19(1)

From:

Miller-Saunders, Kristi

Sent:

November-30-17 9:04 AM

To:

Jones, Simon; Garver, Kyle; Higgins, Mark

Cc:

Taylor, Nathan

Subject:

RE: feedback needed today

I have a call till 10:30, but I will feed into this response.

Kristi

From: Jones, Simon

Sent: November-30-17 8:55 AM

To: Miller-Saunders, Kristi; Garver, Kyle; Higgins, Mark

Cc: Taylor, Nathan

Subject: RE: feedback needed today

All,

To coordinate this request into a single response, please send me your thoughts as bullet points by 11:00.

Thanks,

Simon

Simon R.M. Jones

Acting Division Manager, ADGT

Aquatic Animal Health Section Pacific Biological Station Fisheries and Oceans Canada 3190 Hammond Bay Road Nanaimo, British Columbia V9T 6N7, Canada

Tel: 250 729 8351 Fax: 250 756 7053

E-mail: simon.jones@dfo-mpo.gc.ca

From: Lowe, Carmel

Sent: November-30-17 8:48 AM

To: Miller-Saunders, Kristi; Garver, Kyle; Jones, Simon

Cc: Taylor, Nathan

Subject: feedback needed today

All - can you send me a short summary of the key outcomes/developments/new science of relevance to DFO coming out of the PRV-HSMI workshop this morning?

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Sent from my BlackBerry 10 smartphone on the Rogers network.

No information has been removed or severed from this page

From:

Miller-Saunders, Kristi

Sent:

November-30-17 11:04 AM

To:

Taylor, Nathan

Subject:

FW: BCSFA PRV-HSMI Meeting key points\_Nov 30 2017\_KM.docx

Attachments:

BCSFA PRV-HSMI Meeting key points\_Nov 30 2017\_KM.docx

I meant to CC you on this. Carmel has asked for a summary of key findings from the meeting. As Simon is acting for you, he is compiling.

Kristi

**From:** Miller-Saunders, Kristi **Sent:** November-30-17 11:03 AM

**To:** Jones, Simon; Garver, Kyle (<u>Kyle.Garver@dfo-mpo.gc.ca</u>); Higgins, Mark **Subject:** BCSFA PRV-HSMI Meeting key points\_Nov 30 2017\_KM.docx

Key take-home points from the PRV/HSMI meeting held by the BCSFA

- PRV causation of HSMI is not controversial in Norway.
- In Norway, despite the fact that most farmed fish become positive for PRV in the marine environment, they find that infection of hatchery smolts in freshwater is a risk factor towards the development of more impactful outbreaks of HSMI. In recent years, HSMI outbreaks have occurred as well in freshwater, which tend to cause higher rates of mortality (up to 50%) than typical marine outbreaks (0-5% on average).
- There is evidence around the world that various strains of PRV have also been associated with
  diseases in *Oncorhynchus* species, principally Coho salmon in Chile and Japan, Rainbow trout in
  Norway, Chile and Washington State, and Chinook salmon in BC. In Washington and BC,
  diseases are associated with the same strain of PRV (PRV-Ia) that is causative of HSMI.
- In Norway, HSMI was first observed in 1999, but shifted over two decades from a disease
  impacting only dozens of farms in central Norway to one that has spread throughout the entire
  Norwegian coast, impacting 100's of farms every year. Their scientists felt that they waited too
  long (5 years) to act on properly reporting and tracking this disease, and advised that even if
  HSMI is not presently highly economically impactful in BC, that action to track and limit its
  spread should be taken sooner rather than later.
- The Norwegian scientists believe that PRV/HSMI may be underestimated and may also contribute to co-infection pathologies but not specifically recognized as the cause of death.
- Preliminary vaccine trials have indicated that vaccines can reduce the development of HSMI, but have not yet resulted in complete protection.
- The three main risk factors toward PRV infection resulting in development of HSMI in Norway are: 1) previous outbreak on same farm, 2) PRV positive smolts put onto farms, 3) nearby farm outbreak—can spread large distances, 4) transmission through feces.
- In situ hybridization studies by BC scientists have shown that the PRV-1a strain in BC is tightly linked with the development of HSMI in Atlantic salmon and Jaundice/anemia in farmed Chinook salmon, whereby the virus is spatially localized within the tissue regions and cells that are damaged by each disease.

From:

Miller-Saunders, Kristi

Sent:

November-30-17 11:14 AM

To:

Jones, Simon; Garver, Kyle (Kyle.Garver@dfo-mpo.gc.ca); Higgins, Mark

Cc:

Taylor, Nathan

Subject:

RE: BCSFA PRV-HSMI Meeting key points\_Nov 30 2017\_KM.docx

### I should have added one more bullet:

Mitigation measures being pursued in Norway include: 1) PRV-free smolts, which involves either screening broodfish and use of only low PRV load carriers or use of on land broodstock with no exposure to PRV, 2) Reduce stress and handling, 3) use of functional diets—highly plant-based diets increase inflammatory responses, hence at an early phase of HSMI development, they switch fish to diets higher in fish-oil.

### Kristi

**From:** Miller-Saunders, Kristi **Sent:** November-30-17 11:03 AM

**To:** Jones, Simon; Garver, Kyle (<u>Kyle.Garver@dfo-mpo.gc.ca</u>); Higgins, Mark **Subject:** BCSFA PRV-HSMI Meeting key points\_Nov 30 2017\_KM.docx

From:

Miller-Saunders, Kristi

Sent:

November-30-17 12:02 PM

To:

Brian Riddell

Cc:

DiCicco, Emiliano

Subject:

BCSFA PRV workshop feedback from DFO Scientists\_Nov 30 2011.docx

Attachments:

BCSFA PRV workshop feedback from DFO Scientists\_Nov 30 2011.docx

I thought you might enjoy reading the variances in what individuals got out of the PRV-HSMI workshop. Quite telling actually.

Kristi

s.21(1)(a)

s.21(1)(b)

### PRV/HSMI Workshop Feedback

### **OVERVIEW**

- Information presented at the Workshop emphasised the different relationship between PRV and HSMI that occurs in BC and Norway.
- Evidence for a causal link between PRV and HSMI is weak in BC and strong in Norway
- Several hypotheses focusing on host, virus and/or environmental variables were presented to explain the differences between the Norwegian and BC experiences.
- The relevance to DFO is twofold: 1, potential impacts to wild salmon and 2, occurrence of HSMI-like diseases in farmed Atlantic salmon in BC.
- Research is presently underway to test these hypotheses and to address the knowledge gaps
- detailed observations from DFO attendees are listed below

### MILLER

- PRV causation of HSMI is not controversial in Norway.
- In Norway, despite the fact that most farmed fish become positive for PRV in the marine environment, they find that infection of hatchery smolts in freshwater is a risk factor towards the development of more impactful outbreaks of HSMI. In recent years, HSMI outbreaks have occurred as well in freshwater, which tend to cause higher rates of mortality (up to 50%) than typical marine outbreaks (0-5% on average).
- There is evidence around the world that various strains of PRV have also been associated with diseases in *Oncorhynchus* species, principally Coho salmon in Chile and Japan, Rainbow trout in Norway, Chile and Washington State, and Chinook salmon in BC. In Washington and BC, diseases are associated with the same strain of PRV (PRV-Ia) that is causative of HSMI.
- In Norway, HSMI was first observed in 1999, but shifted over two decades from a disease impacting only dozens of farms in central Norway to one that has spread throughout the entire Norwegian coast, impacting 100's of farms every year. Their scientists felt that they waited too long (5 years) to act on properly reporting and tracking this disease, and advised that even if HSMI is not presently highly economically impactful in BC, that action to track and limit its spread should be taken sooner rather than later.
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- Preliminary vaccine trials have indicated that vaccines can reduce the development of HSMI, but have not yet resulted in complete protection.
- The three main risk factors toward PRV infection resulting in development of HSMI in Norway are: 1) previous outbreak on same farm, 2) PRV positive smolts put onto farms, 3) nearby farm outbreak—can spread large distances, 4) transmission through feces.
- In situ hybridization studies by BC scientists have shown that the PRV-1a strain in BC is tightly linked with the development of HSMI in Atlantic salmon and Jaundice/anemia in farmed

- Chinook salmon, whereby the virus is spatially localized within the tissue regions and cells that are damaged by each disease.
- Mitigation measures being pursued in Norway include: 1) PRV-free smolts, which involves either screening broodfish and use of only low PRV load carriers or use of on land broodstock with no exposure to PRV, 2) Reduce stress and handling, 3) use of functional diets—highly plant-based diets increase inflammatory responses, hence at an early phase of HSMI development, they switch fish to diets higher in fish-oil.

### **MACWILLIAMS**

- In Norway this appears to be a self limiting infection, except for the subpopulation of individuals that experience a type IV hypersensitivity reaction with progression to a disease state with low cumulative mortality
- Norwegian disease outbreak experience typically involves concurrent infections or recent infections (resulting in a sensitized/reactive immune system?)
- Canadian situation self limiting infection, typically asymptomatic, little-to-no apparent clinical relevance from a herd health perspective
- Transmission from an unknown wild marine reservoir, highly contagious (easily spread) within a farm population, with 100% prevalence for infection within 6 months of initial detection
- Lower prevalence in wild Pacific salmon could be due to host response (i.e. species-specific differences in number or types of cellular receptors for viral attachment to erythrocytes) or related to poor viral particle survival outside a host
- Adopting a standard case definition, encompassing both farm level clinical presentation and pathology, would add clarity to future discussions and communications

### GARVER

- Kathleen Frisch, and Espen Rimstad presented on the occurrence of HSMI in Norway. In Norway, the disease HSMI is associated with clinical signs of disease on the farm and is a production concern. However the disease often co-occurs with other diseases or is present with mixed aetiologies, such as CMS, PD which have not been found to be present in BC or USA waters. HSMI rarely occurs as the sole disease in Norwegian aquaculture.
- Lesions characteristic for HSMI have been recreated in Norwegian laboratory studies when fish
  are exposed to purified PRV indicating that PRV is the cause of HSMI. However the clinical
  disease as it occurs in Norwegian Aquaculture has not been recreated in a laboratory setting.
  This suggests that there are environmental or other disease determinate factors that are
  required to get clinical disease. Research is ongoing in Norway, Canada, and the USA to better
  understand the factors required to cause HSMI.
- In Norway, HSMI resistant Atlantic salmon have been development by AquaGen. Fish appear to be less susceptible to the disease however they appear to be equally susceptible to PRV infection, pointing towards the importance of host factors in the development of HSMI disease.

- Moreover, research presented by Drs Mark Polinski and Emiliano DiCicco point towards a plausible hypothesis that HSMI may be a result of a hypersensitivity type reaction whereby host macrophages are activated causing an inflammatory response.
- To date, laboratory studies in Canada have demonstrated that despite high PRV loads in a salmon host, HSMI has not been induced. Minor inflammation has been observed due to the presence of PRV however these lesions have not resulted in any measurable harm to the respiratory physiology of Atlantic salmon.
- PRV is present on the east coast of Canada and is highly prevalence in Atlantic salmon aquaculture where typically prevalence reaches 100% by 6 months post seawater entry. The viral source for these infections remains unknown.
- All Pacific salmonid appear susceptible to PRV infection, however surveillance of wild/free
  ranging stocks suggest Coho and Chinook salmon to be the most susceptible. In farmed Chinook
  in BC, PRV has been associated with a jaundice syndrome and in Washington state, PRV has
  been associated with erythrocytic inclusion body syndrome (EIBS) in hatchery reared coho
  salmon. What role PRV plays in these syndromes remains unknown. Research studies are being
  conducted in the USA to better understand the relationship of PRV and EIBS.
- Collaborative studies are also underway between Norway and Canada that involves side-by-side
  comparisons of the Norwegian –PRV vs. British Columbia-PRV in their ability to cause disease in
  Atlantic salmon. This direct comparison of viruses within one laboratory will allow for an
  evaluation of host, environment and virus factors to be individually measured with the ultimate
  goal of determining the factors responsible for disease development.

### **HIGGINS**

- The PRV/HSMI experience in Norway is very different than that seen in BC. It was clear from presentations given by the Norwegian Researchers that there are multiple disease issues on Norwegian farms that lead to a much higher level of mortality among farms over the course of a production cycle. This is not the case in BC.
- Mitigation strategies in Norway include 1) avoiding stressful situations on farms where HSMI has been detected (i.e. lice treatments) 2) use of PRV negative (screened) eggs if possible (the Norwegians do not remove PRV+ eggs from production), and 3) use of functional diets (higher in fish oils).
- Mortality is most common now in the 1-5% range for HSMI outbreaks (no longer do they see 20%). However, freshwater outbreaks of HSMI have seen mortality as high as 50%
- In Norway, the presence of PRV in fish tends to reduce the incidence of Salmon Alpha Virus (SAV) which can cause much higher mortality and is considered as a reportable disease.
- While HSMI has been reported in BC, the overall impact to farmed Atlantic salmon is minimal.

### Dickie, Catherine

From:

Lowe, Carmel

Sent:

November 30, 2017 12:25 PM

To:

Jones, Simon

Cc:

Taylor, Nathan; Miller-Saunders, Kristi; Garver, Kyle; Higgins, Mark; MacWilliams,

Christine

Subject:

RE: feedback needed today

Follow Up Flag:

Follow up

Flag Status:

Completed

Thanks all - really appreciate your summaries. Lots of fodder in these reports for us to consider as we move forward with our aquaculture research programs

### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Oceans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360

Government of Canada | Gouvernement du Canada

From: Jones, Simon

Sent: Thursday, November 30, 2017 11:53 AM To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>

Cc: Taylor, Nathan < Nathan\_Taylor@dfo-mpo.gc.ca>; Miller-Saunders, Kristi < Kristi.Saunders@dfo-mpo.gc.ca>; Garver,

Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; MacWilliams, Christine

<Christine.MacWilliams@dfo-mpo.gc.ca>

Subject: RE: feedback needed today

Carmel,

As requested, an overview of the PRV/HSMI workshop.

Simon

Simon R.M. Jones Acting Division Manager, ADGT

Aquatic Animal Health Section Pacific Biological Station Fisheries and Oceans Canada 3190 Hammond Bay Road

Nanaimo, British Columbia V9T 6N7, Canada

Tel: 250 729 8351 Fax: 250 756 7053

E-mail: simon.jones@dfo-mpo.gc.ca

From: Lowe, Carmel

Sent: November-30-17 8:48 AM

To: Miller-Saunders, Kristi; Garver, Kyle; Jones, Simon

Cc: Taylor, Nathan

Subject: feedback needed today

All - can you send me a short summary of the key outcomes/developments/new science of relevance to DFO coming out of the PRV-HSMI workshop this morning?

### Carmel

Sent from my BlackBerry 10 smartphone on the Rogers network.

### Ryan, Patricia

From:

Moore, Wayne

Sent:

November-30-17 12:24 PM

To:

Thomson, Andrew

Cc:

McPherson, Arran; Parsons, Jay; Lowe, Carmel; LaRue, Jean-François

Subject:

Pathogen transfer risk assessment

Attachments:

Temp1.xlsx; temp2.xlsx

Andy,

Great chat yesterday.

Attached are the documents we mentioned.

I know Jay was out recently speaking to Corey and Nathan (from Carmel's shop) on this amongst other things. As discussed, I think it would be a great idea for the four of us (you, me, Carmel and J-F) to get collectively briefed on this soon so that we can ensure that the needed resources et al are aligned and that if we see any problems down the road we can flag them now.

Happy to organize if you would find that of value.

W

### Wayne Moore

Director General, Strategic and Regulatory Science Fisheries and Oceans Canada / Government of Canada Wayne.Moore@dfo-mpo.gc.ca / Tel: 613-990-0001

Directeur général, Sciences stratégiques et réglementaires Pêches et Océans Canada / Gouvernement du Canada <u>Wayne.Moore@dfo-mpo.gc.ca</u> / Tél. : 613-990-0001

Web: DFO/MPO

Twitter: DFO/MPO/DFO-Science/MPO-Science

### Systemic bacterial infection risk assessments

To be completed by: Pathogen transfer risk assessment team
Deadline: Jul-18

%, d	lone	Phase	Duc By	Notes (lead)
2	25%	Problem formulation	8-Dec-17	Caroline Mimeault
	0%	Pathogen characterisation	12-Jan-18	Linda Rhodes (NOAA)
	0%	Risk assessment	27-Apr-18	Caroline Mimeault
	0%	Science Advisory Report	6-Jul-18	Ingrid Burgetz and Jay Parsons

Enterio	Redmouth Disease (ER/	
Victorie	Phase	Due By 19 Notes
25%	Problem formulation	8-Dec-17 Caroline Mimeault
0%	Pathogen characterisation	12-Jan-18 Joy Wade (AquaFundy)
0%	Risk assessment	27-Apr-18 Caroline Mimeault
0%	Science Advisory Report	6-Jul-18 Ingrid Burgetz and Jay Parsons

Fil	iranc	ulosis		
9/6.	done	Phase	Due By	Notes
	25%	Problem formulation	8-Dec-17	Caroline Mimeault
	0%	Pathogen characterisation	12-Jan-18	France Boily
	0%	Risk assessment	27-Apr-18	Caroline Mimeault
	0%	Science Advisory Report	6-Jul-18	Ingrid Burgetz and Jay Parsons

Salmoi	nid Rickettsial Septicaem	ia (SRS)
% done	Phase	Due By Notes Transfer and Trans
25%	Problem formulation	8-Dec-17 Caroline Mimeault
0%	Pathogen characterisation	12-Jan-18 Simon Jones
0%	Risk assessment	27-Apr-18 Caroline Mimeault
0%	Science Advisory Report	6-Jul-18 Ingrid Burgetz and Jay Parsons

Pathogen Transfer Risk Assessments in the Discovery Islands (bacteria causing systemic infections)

Commonante	400	- G - C	Commission	Bacterial Kidney Disease (BKD)	sease (BKD)	Enteric Redmouth (ERM)	th (ERM)	Furunculosis	sis	Salmonid rickttsial septicaemia (SRS)	oticaemia (SRS)	
mponents	danc	200	Compresed Or	Lead	Done (%)	Lead	Done (%)	Lead	Done (%)	Lead	Done (%)	Notes
	Problem formulation (draft)	10-Nov-17	10-Nov-17	Caroline	100	Caroline	100	Caroline	001 海馬	Caroline	100	
	Meeting with client	10-Nov-17		Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	
Problem	Engage with CFIA	10-Nov-17		Wayne/Jay	0	Wayne/Jay	0	Wayne/Jay	0	Wayne/Jay	0	
formulation	Working group formation	17-Nov-17		Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	
	Working group meeting	30-Nov-17		Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	
	Problem formulation (final)	8-Dec-17		Caroline	0	Caroline	0	Caroline	0	Caroline	0	
	Update industry	TBD		Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	
Communications	Debrief NGOs, First Nations, province	TBD		Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	0	Jay/Ingrid	9	
	Case definition	18-0ct-17	1-Nov-17	lan/Caroline/France	8	lan/Caroline/France	100	lan/Caroline/France	100	lan/Caroline/France	100	
_	Contact industry	20-0ct-17	2-Nov-17	Jay		Jay	300	Jay	100	Jay	100	
Data	Industry data acquisition	10-Nov-17		Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	
	Industry data analysis	26-Jan-18		Caroline/France/epi?	0	Caroline/France/epi?	0	Caroline/France/epi?	0	Caroline/France/epi?	0	
	Outlines	2-0ct-17	2-0ct-17	Caroline	700	Caroline		Caroline	100	Caroline	001100	
	Identification of lead author	2-0ct-18	2-0ct-18	Ingrid/Caroline	100	Ingrid/Caroline		Ingrid/Caroline	100	Ingrid/Caroline	001	
Dath agent and ded	Pathogen working paper (draft)	12-Jan-18		Linda Rhodes	0	Joy Wade	0	France Boily	10	Simon Jones	Q	
CHOSEH WOLVING	Distribution for peer review	4-May-18		Caroline	0	Caroline	0	Caroline	0	Caroline	0	
habei	Pathogen working paper (reviewed)			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0	
	Pathogen working paper (approval)			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0	
	Pathogen working paper (final)	1-Sep-18		Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0	
	Risk assessment workshop	2-Feb-18		Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	
	Risk assessment (draft)	16-Mar-18		Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	
Risk assessment	Distribution for peer review	4-May-18		Caroline	0	Caroline	0	Caroline	0	Caroline	0	
working paper	Risk assessment (reviewed)			Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	
	Risk assessment (approval)			Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	
	Risk assessment (final)	1-Sep-18		Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	
	Meeting agenda	4-May-18		Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0	
	Distribution of meeting material	4-May-18		Steering committee	0	Steering committee	0	Steering committee	0	Steering committee	0	
CGAS	Presentation for pathogen paper	15-Jun-18		Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0	
COOL SOCIOIS	Presentation for risk assessment	15-Jun-18		Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0	
I-review	CSAS peer-review meeting	15-Jun-18		CSAS meeting chair	0	CSAS meeting chair	0	CSAS meeting chair	0	CSAS meeting chair	0	
process	Reviewed pathogen paper			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0	
	Reviewed risk assessment			Caroline	0	Caroline	0	Caroline	0	Caroline	0	
	Draft SAR			CSAS participants	0	CSAS participants	0	CSAS participants	0	CSAS participants	0	
	Revised SAR			Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0	
	Approval of pathogen paper			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0	
	Approval of risk assessment			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0	
	Approval of SAR			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0	
Deliverables	Translation of SAR			CSAS office	0	CSAS office	0	CSAS office	0	CSAS office	0	
	Translation of figures included in SAR			Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0	
	Submission of pathogen paper	1-Sep-18	1-Sep-18	Jay	0	Jay	0	Jay	0	Jay	0	
	Submission of risk assessment	1-Sep-18	1-Sep-18	Jay	0	Jay		Jay	0	Jay	0	
	Submission of SAR (English)			Jay	0	Jay		Jay	0	Jay	0	
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Best available copy

ESTIMATED TIMELINES FOR PATHOGEN TRANSFER RISK ASSESSMENTS IN THE DISCOVERY ISLANDS, BC (reviewed on October 2, 2017)

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ESTIMATED TIMELINES FOR PATHOGEN TRANSFER RISK ASSESSMENTS IN THE DISCOVERY ISLANDS, BC (reviewed on October 19, 2017)

Risk assessment Pathogen phase	Pathogen	Disease	71-qa2	71-300	TI-VON	Dec-17	81-de4	Mar-18	81-1qA	81-ysM	81-nuc	81-lut	81-guA	St-q92 8r-150	81-voM	81-39G	er-nst	Feb-19	er-1sM	er-19A	er-ysM	et-nuc	er-Inc	61-guA	Sep-19	61-15O	el-von	Dec-19	าสท-20	Feb-20	Mar-20	Apr-20	02-ysM	02-unc	Jul-20	02-guA	02-dəS
Virus	Viral hemorrhagic septicaemia virus	Viral hemorrhagic septicaemia (VHS)			+ + + + + + + + + + + + + + + + + + +	11 A A A A A A A A A A A A A A A A A A				4.6																										O	Cohen
	Aeromonas salmonicida	Furunculosis	200-721-731-731 201-731-731-731 201-731-731-731	ar Jaray Janasa Dr. Ula Brahasa Dr. Ula Brahasa			1005		2 8 2 8 2 5 3 2 2 5 3 2	S.E.S.S				194																						O	Cohen
Bacteria causing	Piscirickettsia salmonis	Salmonid rickettsial septicaemia (SRS)							2 2 15 3 2 15 3 5 5	A Audio			244																							O	Cohen
systemic infections	Renibacterium salmoninarum	Bacterial kidney disease (BKD)		8-0-6-V			2.5 1.6 2.6 1.6 2.6 1.6		A TE TO SE				1411	3.114																						U	Cohen
	Yersinia ruckeri	Enteric redmouth disease (ERM)				100 (E.A.) (C.A.) (C.A.) (C.A.) (C.A.) (C.A.)	A 1.6.1 3.20.5 3.4.4	E (2.8)			0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	20 数差子。 数据学3 20 多的	2 de 2-3 de 26 de 2-3 de																							O	Cohen
Bacteria causing	Moritella viscosa	Winter ulcers												••••			2 5 3 4 5 1 2 5 3 4 5 1	2.6 8.3 3	2,2,2,3,3																	O	Cohen
erosive lesions	Tenacibaculum maritimum	Mouth rot									2 5 5 2 5 5 2 6 5 4			1945 1945 1886				2 2 E 9		學為選集2																O	Cohen
Parasite	Parameoba perurans	Parameoba perurans Amoebic gill disease												3.5 3.5	97 a 32 b 47 a 47 a			2 2 3 2 3 3 2 3 3 2 3 3 2	F T A.	6.85 S S S S 6.85 S S S S	*****************	3 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	* * * * * * * * * * * * * * * * * * *													O	Cohen
Others	Idiopathic heart disease (will also consider HSMI and PRV publications)	se (will also consider ations)														9.6		1 39 .34 3 .35 9 4 80 000	34.44 4.5 5.5	# 10 A 2 A 3 A 3 A 3 A 3 A 3 A 3 A 3 A 3 A 3	8 H   8 H	5. 在 元 3 4 5 5. 在 元 3 4 5 5. 在 元 3 4 5	12			9.4 9.0	38 N 5 e 6 h									O	Cohen
Synthesis	Pathogens that caused disease on Atla salmon farms in the Discovery Islands	Pathogens that caused disease on Atlantic salmon farms in the Discovery Islands																				**************************************	1		3 UF 6	. 1146 Ar 1.308 A.113 41.5	DA. BERT		N 9 4-25-35	##16-E	Sarya.	2 (1.55 to 1.55 to 1.5	12 ( 13 ( 13 ( 13 ( 13 ( 13 ( 13 ( 13 (			O	Cohen

### Ryan, Patricia

From:

Moore, Wayne

Sent:

November-30-17 3:57 PM

To:

Lowe, Carmel; McPherson, Arran; Taylor, Nathan; Parsons, Jay

Subject:

RE: Request for feedback

Fabulous. Thanks.

From: Lowe, Carmel

Sent: November 30, 2017 3:26 PM

To: McPherson, Arran <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran.McPherson@dfo-mpo.gc.ca</a>; Moore, Wayne <a href="mailto:Wayne.Moore@dfo-mpo.gc.ca">Wayne.Moore@dfo-mpo.gc.ca</a>; Taylor,

Nathan < Nathan.Taylor@dfo-mpo.gc.ca >; Parsons, Jay < Jay.Parsons@dfo-mpo.gc.ca >

Subject: Request for feedback

fyi

### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique

3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

### Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360 Government of Canada | Gouvernement du Canada

From: Jones, Simon

Sent: Thursday, November 30, 2017 11:53 AM

To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>

Cc: Taylor, Nathan < Nathan. Taylor@dfo-mpo.gc.ca >; Miller-Saunders, Kristi < Kristi. Saunders@dfo-mpo.gc.ca >; Garver,

Kyle < Kyle.Garver@dfo-mpo.gc.ca >; Higgins, Mark < Mark.Higgins@dfo-mpo.gc.ca >; MacWilliams, Christine

<Christine.MacWilliams@dfo-mpo.gc.ca>

Subject: RE: Request for feedback

Carmel,

As requested, an overview of the PRV/HSMI workshop.

Simon

Simon R.M. Jones Acting Division Manager, ADGT Aquatic Animal Health Section Pacific Biological Station Fisheries and Oceans Canada 3190 Hammond Bay Road Nanaimo, British Columbia V9T 6N7, Canada

Tel: 250 729 8351 Fax: 250 756 7053

E-mail: simon.jones@dfo-mpo.gc.ca

From: Lowe, Carmel

Sent: November-30-17 8:48 AM

To: Miller-Saunders, Kristi; Garver, Kyle; Jones, Simon

Cc: Taylor, Nathan

**Subject:** Request for feedback

All - can you send me a short summary of the key outcomes/developments/new science of relevance to DFO coming out of the PRV-HSMI workshop this morning?

Carmel

Sent from my BlackBerry 10 smartphone on the Rogers network.

### Pages 1690 to / à 1691 are withheld pursuant to sections sont retenues en vertu des articles

14(a), 21(1)(b), 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

### McLeod, Patricia

From:

Miller-Saunders, Kristi

Sent:

December 5, 2017 4:10 PM

To:

Taylor, Nathan

Subject:

RE:

From: Taylor, Nathan

Sent: December 5, 2017 3:54 PM

To: Miller-Saunders, Kristi

Subject: (

Hi Kristi,

Thanks!

Nathan

Nathan G. Taylor, Ph.D.

Division Manager | Directeur de secteur

Aquatic Diagnostics Genomics and Technology Division | Division des diagnostics, la genomique, de la technologie aquatique Fisheries and Oceans Canada | Peches et Oceans Canada Pacific Biological Station | Station biologique du Pacifique

250-756-7395

s.21(1)(a)

s.21(1)(b)

s.23

Parsons, Jay Taylor, Nathan From: Sent: Tuesday, December 05, 2017 7:05 PM To: Marty, Gary D AGRI:EX Creative Salmon'; - Creative Salmon'; Cc: Parsons, Jay Subject: Thanks for this. s.14(a) s.21(1)(a) I'll be back in touch regarding what is said when I have news. s.21(1)(b) s.23 Best Nathan

### Page 1694 is withheld pursuant to sections est retenue en vertu des articles

14(a), 21(1)(b), 23, 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

From:

Miller-Saunders, Kristi

Sent:

December-06-17 9:19 AM

To:

pac.prmc / pac.urpcm (DFO/MPO)

Subject:

RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

Actually I see that you did address the monitoring program, so you can leave it as is. However, you may want to talk to Corey about any corrections or additions.

\*\*\* Regarding your statement about Dr. Miller-Saunders February 2017 research, the Department prides itself on maintaining an objective science research program, focused on DFO's priority issues. Results of this research are peer reviewed and published in international scientific journals. DFO scientists take into account all peer-reviewed science of which they are aware. DFO's monitoring programs evolve/are updated as research identifies new methodologies that offer improved results.

### Kristi

From: pac.prmc / pac.urpcm (DFO/MPO)

Sent: December 5, 2017 6:17 PM

To: Miller-Saunders, Kristi

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

Would you be able to edit the text so it is correct and addresses the implication, please?

----Original Message-----

From: Miller-Saunders, Kristi

Sent: Tuesday, December 05, 2017 6:14 PM To: pac.prmc / pac.urpcm (DFO/MPO)

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

Yes I think that would work. However the audit program is not a research program so if you are responding to an implied criticism made in my paper on our regulatory program, which was really placed there to explain why HSMI was not diagnosed previously through this program, I am not sure that is covered in your research statement.

From: pac.prmc / pac.urpcm (DFO/MPO)

Sent: December 5, 2017 5:44 PM

To: Miller-Saunders, Kristi

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

HI Kristi,

In the draft, I have this paragraph:

The Department prides itself on maintaining an objective science research program, focused on DFO's priority issues. Results of this research are peer reviewed and published in international scientific journals. DFO scientists take into account all peer-reviewed science of which they are aware.

\*could we say something like the text below, after the \*\*\*? I'm unsure how much we can add re HSMI, since we've been told not to add to our standard lines since they and the website offer a complete response, BUT if you feel a direct reference is appropriate,

s.21(1)(a)

1 s.21(1)(b)

\*\*\* Regarding your statement about Dr. Miller-Saunders February 2017 research, the Department prides itself on maintaining an objective science research program, focused on DFO's priority issues. Results of this research are peer reviewed and published in international scientific journals. DFO scientists take into account all peer-reviewed science of which they are aware. DFO's monitoring programs evolve/are updated as research identifies new methodologies that offer improved results.

Thanks Candace.

----Original Message-----From: Miller-Saunders, Kristi

Sent: Tuesday, December 05, 2017 5:11 PM To: pac.prmc / pac.urpcm (DFO/MPO)

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

I will review what the paper said but we would have never suggested the program was "faulty" but I believe we did point out that it was not designed to recognize or track emerging disease issues but rather was designed to ensure compliance for reportable diseases. Moreover, while the program does track common easily recognized endemic diseases it does not have any built in measures to follow up on lesion patterns that do not fit those from well characterized diseases. The only aspect i suppose that may be referred to as faulty was the lack of inclusion of skeletal muscle or pancreas tissues which are required to differentiate HSMI from pancreas disease or cardiomyopathy syndrome. This was pointed out by the pathologist from tge sshi who teach the slides from 2011 to 2013. From 2013 onward these tissues were included.

Kristi

From: pac.prmc / pac.urpcm (DFO/MPO)

Sent: December 5, 2017 4:59 PM

To: Miller-Saunders, Kristi

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

Hi,

I was just letting you know what our standard lines are on PRV (the material inside the quotation marks). That included the website link. We were told the website would be updated as necessary with approved lines (I see it was last updated May 26).

I just need any input you might have re their comments (bottom of page 4) that your Feb 2017 paper criticizes "the faulty scientific approaches" used in the Fish Health and Surveillance Program.

I've drafted the response and am about to send it to AQ staff for review. I've left a placeholder for your input.

Thanks Candace

----Original Message----

From: Miller-Saunders, Kristi

Sent: Tuesday, December 05, 2017 4:34 PM

To: pac.prmc / pac.urpcm (DFO/MPO)

Subject: RE: \*\* INPUT NEEDED\*\* URGENT \*\*: Incoming 2017-001-02372

Hello Candace,

s.21(1)(a)

s.21(1)(b)

s.23

will try to take a look at this material tonight. Not sure why you are pointing me to the website as I was one of the scientists who contributed to it. It becomes out of date rapidly however as scientists continue to study this issue.
Kristi
From: pac.prmc / pac.urpcm (DFO/MPO) Sent: December 5, 2017 3:29 PM To: Miller-Saunders, Kristi Subject: ** INPUT NEEDED** URGENT **: Incoming 2017-001-02372
Hi Kristi I'm working on a draft and will be talking to Corey about our approach. But in the meanwhile, I'm specifically looking for any input you might have re their comments (bottom of page 4) that your Feb 2017 paper criticizes "the faulty scientific approaches" used in the Fish Health and Surveillance Program.
I don't need input re PRV (the website includes a reference to your 2017 study): "Regarding your comments regarding heart and skeletal muscle inflammation (HSMI) and piscine reovirus (PRV), DFO's website <a href="http://www.dfo-mpo.gc.ca/science/aah-saa/species-especes/aqhealth-sante/prv-rp-eng.html">http://www.dfo-mpo.gc.ca/science/aah-saa/species-especes/aqhealth-sante/prv-rp-eng.html</a> has detailed and up-to-date information. I strongly encourage you to read this material, which provides the scientific and historical context of this complex issue."
I need your input asap
Thanks Candace

s.21(1)(a)

s.21(1)(b)

s.23

Miller-Saunders, Kristi Miller-Saunders, Kristi From: Sent: December-07-17 11:12 PM To: Thomson, Andrew Subject: RE: recent science in the news What about the 18th? From: Thomson, Andrew Sent: December 7, 2017 10:22 PM To: Miller-Saunders, Kristi Subject: Re: recent science in the news Hi Kristi. back the following. Might be able to find a short time tommorow Andrew J L Thomson Regional Director | Directeur Régionale Fisheries Management Branch | Direction de la gestion des pêches Pacific Region | Region du Pacifique Fisheries & Oceans Canada | Pêches et Océans Canada Suite 200 - 401 Burrard St. Vancouver, BC, Canada V6C 3S4 andrew.thomson@dfo-mpo.gc.ca Telephone | Téléphone 604.666.0751 Facsimile | Télécopieur 250.666.8069 Government of Canada | Gouvernement du Canada. From: Miller-Saunders, Kristi Sent: Thursday, December 7, 2017 9:38 PM To: Thomson, Andrew Subject: recent science in the news Hello Andrew, We really should talk about our recent SSHI findings, the Creative salmon study, and the recent industry meeting. Are you around next week at all?

Kristi

s.19(1)

s.21(1)(a)

s.21(1)(b)

From: Sent: To:	Miller-Saunders, Kristi December-07-17 1:14 PM Tabata, Amy
Cc: Subject:	Taylor, Nathan RE: Field sampling.
and was standing on all the	NEI Tela sampling.
-	that question is that our lab, with collaboration with Curtis marine virology lab, is the with water samples. Moreover our PRV test has been diagnostocally validated with our yses.
They came to us as the diagnos	tic labs said they could not process water samples.
Kristi	
From: Tabata, Amy Sent: December 7, 2017 11:03 A To: Miller-Saunders, Kristi Subject: Field sampling.	AM
Hi Kristi.	
An update on the sampling this	week
plants in this time, and collecte	nada and BC OE personnel From Dec 4-6. We visited and inspected 2 fish processing d samples for their routine analysis as well as for viral/microbe analysis. A portion was ogen and -80), and the rest stored at 4C and further stored or processed within 72 hours.
Salmon. As such, Cre was involved, and not the fish hassist, the 2 other organizations right now,	the second site we visited, Lions Gate Fisheries in Tofino, is partnered with Creative ative salmon was at the second meeting with them, and was questioning why our lab Health lab/ veterinarians. I could only say that I was there at the request of, and to s, and this was not a DFO-led initiative. Given the sensitivity and the huge public profile  I talked with Nathan briefly se of where I had been and what was happening. He asked for a brief description of o, which I provided.
Amy	

s.19(1)

s.21(1)(b)

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-08-17 4:57 PM

To:

Parsons, Jay

Subject:

RE: Pathogen transfer risk assessment

This is great. I assume that it means you will be going to Vancouver for next Thursday? Or will we do it by phone. I want Caroline to be there and I want one of you or Ingrid to be here.

I can't afford to have both of you offsite.

From: Parsons, Jay

Sent: December 8, 2017 4:33 PM

To: Moore, Wayne < Wayne. Moore@dfo-mpo.gc.ca>

Subject: Pathogen transfer risk assessment

Importance: High

Wayne,

I want to provide an update and next steps on the pathogen Risk Assessments.

For the meeting with Carmel, JF, Andy, and yourself, we have shared the Gantt chart with the timelines for all remaining pathogens and the detailed work plan for the next four risk assessments. I have also attached a table that shows the leads for all nine risk assessments and details on the composition of the risk assessment working group team. Please let me know if additional materials are required for the meeting.

In addition, most of the key elements are in place but a couple of key gaps exist. In terms of what is in place, we have the leads for the pathogen papers and the risk assessments identified, although there are a couple of gaps for leads on some of the bacterial pathogen papers that we will need to contract externally as this expertise does not exist in the Pacific Region. As well that is great news that CFIA is onside to continue to provide risk assessment support. And we are working closely with the BCSFA to access the industry fish health data. And we continue to have the support of the Pacific Region fish health researchers and oceanographers.

But one key area we require input and expertise is around salmon population modelling expertise and approaches to support the consequence assessments for the remaining risk assessments and the overall synthesis piece.

When I was in the Pacific Region a few weeks ago discussing this and other aquaculture and genomics topics with Nathan, he brought John Holmes (Division Manager of salmon group) into the discussion on the risk assessment to start the discussion on what is needed, so that the appropriate regional experts can be identified and brought into the risk assessment team. And yesterday, I organised a call with Nathan, John and Eddy Kennedy, to make progress on identifying the salmon population and ecology experts (including ecological modelers). It quickly became apparent that in order to make progress on identifying the right staff and articulating the best modelling approach for the individual risk assessments as well as the synthesis piece that a two part face to face meeting is needed in short order to elaborate what is required from a risk assessment perspective, what salmon population modelling can offer, and what approaches and expertise can be brought to bear to support the initiative. Based on the input from Nathan, Eddy and John the only opportunity to hold this meeting is next Thursday at PBS. I have attached a draft agenda for the meeting that we are discussing with them.

As you and others have noted, this is a high priority initiatives and timelines are tight for delivering all the advice and there is significant work that needs to be completed, and we are at a critical stage that the immediate identification of experts and approaches is required for us to continue to meet the aggressive timelines that we have laid out for the next four risk assessments (as well as the remaining ones).

s.21(1)(a)

With regards to the next four assessment, we have a deadline of end of December/early January for receiving the draft pathogen papers (we have already received one) and these are required for us to start drafting the risk assessment papers in early January (along with the support of salmon population modelling outputs). So we are endeavouring to maintain our timelines for a June 2018 CSAS review of the next four risk assessments.

Please let me know if you have any questions or concerns with the update or approaches.

Jay

## Ryan, Patricia

From:

Moore, Wayne

Sent:

December-11-17 8:34 AM

To:

Parsons, Jay

Subject:

Re: Pathogen transfer risk assessment

Thx

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Parsons, Jay

Sent: Monday, December 11, 2017 8:17 AM

To: Moore, Wayne

Subject: RE: Pathogen transfer risk assessment

The is the memo you, Carmel, Andy and JF signed-off on recently that we can share or as you say verbal briefing.

Jay

From: Moore, Wayne

Sent: Saturday, December 09, 2017 10:39 AM

To: Parsons, Jay

Subject: RE: Pathogen transfer risk assessment

Great – I think these are all the right documents. I wonder if we have on the shelf a briefing note or a deck that explains the background a bit (or alternatively I assume we can do verbally as most everyone knows). It would be good to get any assumptions we have made on the table.

From: Parsons, Jay

Sent: December 8, 2017 4:33 PM

To: Moore, Wayne < Wayne. Moore@dfo-mpo.gc.ca>

Subject: Pathogen transfer risk assessment

Importance: High

Wayne,

I want to provide an update and next steps on the pathogen Risk Assessments.

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As you and others have noted, this is a high priority initiatives and timelines are tight for delivering all the advice and there is significant work that needs to be completed, and we are at a critical stage that the immediate identification of experts and approaches is required for us to continue to meet the aggressive timelines that we have laid out for the next four risk assessments (as well as the remaining ones).

With regards to the next four assessment, we have a deadline of end of December/early January for receiving the draft pathogen papers (we have already received one) and these are required for us to start drafting the risk assessment papers in early January (along with the support of salmon population modelling outputs). So we are endeavouring to maintain our timelines for a June 2018 CSAS review of the next four risk assessments.

Please let me know if you have any questions or concerns with the update or approaches.

Jay

From:

Miller-Saunders, Kristi

Sent:

December-11-17 11:37 AM

To:

Taylor, Nathan

Subject:

FW: Fish farm update

FYI I need to respond to this ASAP. What would you like me to say?

From: Hunse, Laura A ENV:EX [mailto:Laura.Hunse@gov.bc.ca]

Sent: December-11-17 11:10 AM

To: Austin, Joyce ENV:EX; Miller-Saunders, Kristi

**Cc:** Freyman, Liz ENV:EX **Subject:** RE: Fish farm update

Hi joyce, i can't answer some of those. Brady Nelless (Compliance Director) asks that questions be directed to him so we can stay on top of all info coming and out from one place. i believe he is sending out an email with this info very shortly, in the meantime, I have forwarded your email to him.

I see Amy has already responded to you with the information she does have and is directing further inquiries to Kristi Miller.

Thanks, Laura

From: Austin, Joyce ENV:EX

**Sent:** Monday, December 11, 2017 10:49 AM **To:** Kristi Miller (<u>Kristi.Miller@dfo-mpo.gc.ca</u>) **Cc:** Freyman, Liz ENV:EX; Hunse, Laura A ENV:EX

Subject: FW: Fish farm update

Importance: High

Hi Kristi

I have the Minister's office asking me some questions that need answer before noon. Laura is trying to help but can you please weigh in as well?

Thank you,

## Joyce Austin, Ph.D.

Senior Provincial Laboratory Specialist (Unit Head)
Environmental Monitoring, Reporting & Economics

Knowledge Management Branch | Ministry of Environment & Climate Change Strategy

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Cel.:

Fax: 250-356-7197

From: McGuire, Jennifer ENV:EX

**Sent:** Monday, December 11, 2017 10:11 AM **To:** Tesch, David ENV:EX; Graham, Tessa ENV:EX

Cc: Morel, David P ENV:EX Subject: FW: Fish farm update

Need some fact checking on the highlighted bits – pls – need confirmed before noon.

- Results have come back confirming presence of PRV?? True or false? When do we expect the results?

## These are EPD questions:

- If TRUE what does that mean? What does the ministry need to do? Can the discharge be treated? How?
- What is the natural occurrence/presence of PRV in the environment?

Thanks JLM

From: Zacharias, Mark ENV:EX

Sent: Monday, December 11, 2017 9:58 AM

**To:** McGuire, Jennifer ENV:EX **Subject:** FW: Fish farm update

Regards, Mark

From: Zacharias, Mark ENV:EX

Sent: Monday, December 11, 2017 8:23 AM

To: Heyman, George ENV:EX

Cc: Frampton, Caelie ENV:EX; Xia, Eveline ENV:EX; Morel, David P ENV:EX; Salkus, Beverley ENV:EX

Subject: Fish farm update

Minister:

## Inspections and testing:

Ministry compliance staff conducted site visits to the both the Browns Bay Packing (Campbell River) and Lions Gate Fisheries (Tofino) facilities the week of December 4, 2017. The facilities were inspected and samples were collected at both facilities. The blood they are releasing was tested and shows presence of PRV. The samples are currently undergoing lab analysis at the DFO Pacific Biological Station Lab in Nanaimo, and once the results are received, likely this week, they will be reviewed to inform next steps. The Inspection reports will be completed once we have the lab results.

Ministry staff will increase inspections at fish processing plants as part of an upcoming Audit of the sector. This includes reviewing permit requirements and making recommendations for amendments where required, as well as ensuring all regulatory requirements are met and the environment is protected. The scope and timing for the fish processing sector audit are currently being finalized and it is anticipated the results of the audit will be ready for release spring of 2018.

There are approximately 35 Fish Processing Plants (wild & farmed) in BC and there are 35 waste discharge authorizations issued under the Environmental Management Act (EMA) for fish processing plants in BC

The Ministry is aware of the samples taken	but results have not been provided to us. Ministry
compliance staff have recently taken samples as part of an inspection and are awaiting results.	

## Regulatory review and change:

Staff are currently identifying what steps are required to amend fish processing plant waste discharge permits under the Environmental Management Act to require the use of best available technology. Similarly, staff are reviewing the steps required to ensure best practices are mandated for sea lice treatment under the Integrated Pest Management Act.

Regards,

Mark Zacharias | Deputy Minister, Environment BC Ministry of Environment and Climate Change Strategy 5th Floor, 2975 Jutland Road | Victoria, BC | V8W 9M1 |



s.16(2)(c)

s.19(1)

From:

Miller-Saunders, Kristi

Sent:

December-11-17 12:17 PM

To:

Taylor, Nathan

Subject:

Release of talk I gave to Industry

The Provincial Environment agency would like me to provide a copy of the talk I gave at the PRV workshop with industry. Any reason this should be a problem?

Kristi Miller-Saunders, PRD

Head, Molecular Genetics Pacific Biological Station 3190 Hammond Bay Rd Nanaimo BC V9T 6N7 250-756-7155

Kristi.Saunders@dfo-mpo.gc.ca

From:

Miller-Saunders, Kristi

Sent:

December-11-17 12:53 PM

To:

Taylor, Nathan

Subject:

RE: bullets?

Preliminary processing to concentrate the viral RNA from the water has been done, but RNA extractions and qPCR has not.

Kristi

From: Taylor, Nathan

**Sent:** December-11-17 12:50 PM

To: Miller-Saunders, Kristi

Subject: bullets?

## This about right?

- recent film showing fish blood being released into B.C. waters from two fish processing plants, and subsequent testing of these samples at the Atlantic Veterinary College showing that the effluent contains Piscine Reovirus (PRV) produced a follow-up request for analysis by DFO Science.
- Environment and Climate Change Canada as well as the provincial Ministry of Environment & Climate Change Strategy requested DFO's Molecular Genetics lab to apply their molecular assay for PRV to newly collected environmental samples at Brown's Bay Packing in Campbell River and Lion's Gate Fisheries in Tofino in order to determine the presence of absence of PRV in the effluent. The Section Head for the Molecular Genetics Group agreed to do this testing in consultation with the UBC's Marine Virology and Microbiology laboratory.
- The requests came from Ken Russell, Senior Enforcement Officer Enforcement Branch Environment and Climate Change Canada, and Laura Hunse, and Environmental Protection Officer, Compliance Section, Environmental Protection Division, Ministry of Environment and Climate Change Strategy (BC).
- The samples were collected on Dec.4 2017 at the Brown's Bay Packing Co, in Campbell River and on Dec 5-6 at Lion's Gate Fisheries, in Tofino BC.
- The samples have not yet been processed, and the results have not been reported back the requestors.
- The Deputy Minister of the BC Ministry of Environment and Climate Change Strategy is eager to know the timeline for the results

Nathan G. Taylor, Ph.D.

Division Manager | Directeur de secteur

Aquatic Diagnostics Genomics and Technology Division | Division des diagnostics, la genomique, de la technologie aquatique

Fisheries and Oceans Canada | Peches et Oceans Canada Pacific Biological Station | Station biologique du Pacifique 250-756-7395

s.19(1)

From:

Brian Riddell < briddell@PSF.CA>

Sent:

December-11-17 7:56 PM

To:

Miller-Saunders, Kristi

Subject:

RE: Jaundice in situ talk to BCSFA

Sorry, I should also have said t

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

**Sent:** December 11, 2017 7:51 PM **To:** Brian Riddell < briddell@PSF.CA > **Subject:** Jaundice in situ talk to BCSFA

We also gave a similar talk on HSMI. I need to make it small enough to send. However, the BC department of Environment has asked for a copy of this talk, which I discussed with them in relation to the work we are doing on the bloodwater. They wanted to know what we knew about risk to wild salmon.

Kristi

From:

Miller-Saunders, Kristi

Sent:

December-11-17 9:10 PM

To:

Lowe, Carmel

Subject: Attachments: FW: PRV Sampling BCSFA Jaundice Talk KM-ED Nov 28, 2017 Shortened-Comp.pptx

Here is what I sent to the BC Ministry of the Environment. Before we release the PRV testing results, I will have a conversation with them about how they might use this information in their aquaculture review and whether DFO can be of any further assistance, as requested.

Kristi

From: Miller-Saunders, Kristi

Sent: December 11, 2017 8:47 PM

To: Austin, Joyce ENV:EX; Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC)

Subject: RE: PRV Sampling

Joyce,

Enclosed is the talk I gave at a BC Salmon Farmers Association meeting on PRV-HSMI state of knowledge workshop December 4th- 5th attended by Norwegian scientists, industry vets and leaders, BC and US Scientists, and DFO regulators. The talk outlines the most recent research out of my lab on PRV and linkages with disease in Pacific salmon. I apologize that it is long and pretty scientific, but they key points are:

We have demonstrated that PRV infections in Chinook salmon can induce a host response that we have shown previously to be diagnostic of the presence of viral disease. This work was published in Conservation Physiology this year.

We demonstrate that 14% of moribund/dead farmed Chinook salmon on the west coast obtained though the DFO audit program were diagnosed with jaundice/anemia, a disease that around the world has been associated with various strains of PRV. There is only a single strain of PRV in BC, that which is known to cause HSMI in Atlantic salmon. We published on HSMI in BC farmed Atlantic salmon in Feb. 2017 in PlosOne.

We show that throughout the developmental pathway of jaundice and across multiple affected tissues, PRV is localized within the regions and cells that become diseased, whether disease is through cell death (necrosis) in liver and kidney or inflammation in heart. We gave a similar talk on HSMI in Atlantic salmon and also demonstrated PRV localized with inflammatory lesions in heart and skeletal muscle tissue.

The primary infective tissue for PRV in both species is the red blood cells (which is why blood water from farmed fish is potentially a strong risk for PRV transmission to wild fish). We show that while PRV remains exclusively in the blood, even at high levels, it is tolerated and there is no disease response in the host. When the virus leaves the blood cells to infect other tissues/cells, it induces a disease response in the host.

The difference between HSMI in Atlantic salmon and jaundice/anemia in Chinook salmon is that in HSMI, PRV appears to leave the red blood cells without lysing (rupturing) them, whereas in Chinook salmon, there is massive lysis of red blood cells leading to anemia (pale gills and tissues) and overloading the kidney and liver with Heme from the breakdown of hemoglobin. Heme is processed in kidney and liver, but becomes toxic at high levels, leading to necrosis (death) of kidney tubules and hematocytes (liver cells), and a jaundice (yellowing) appearance in the fish. While we show that the virus also directly infects these cells, we suspect the heme overload, caused by PRV lysis of red blood cells, is likely the main mechanism leading to disease in jaundice fish. Liver and kidney are not highly affected in HSMI in Atlantic salmon, as the virus goes on to infect muscle cells (heart and skeletal) causing inflammation. This inflammatory response is present, but much reduced in Chinook salmon with jaundice.

We have also demonstrated early (jaundice) disease development in wild Chinook salmon. There was a presentation by Dr. Maureen Purcell at the same meeting that showed an association of the same strain of PRV with a similar disease, which they and the Japanese call EIBS, in Washington State Coho salmon.

I hope this helps in your consideration of the potential for risk in the release of blood water. I am happy to discuss these results directly if there is any need for clarification. We are working up a publication on these data at present.

Kristi Miller-Saunders Head, Molecular Genetics Pacific Biological Station

From: Austin, Joyce ENV:EX [Joyce.Austin@gov.bc.ca]

Sent: December 11, 2017 10:58 AM

To: Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

Hi Amy,

I have already contacted Kristi Miller and I'm waiting on her to give me a call back,

Thanks

## Joyce Austin, Ph.D.

Senior Provincial Laboratory Specialist (Unit Head) Environmental Monitoring, Reporting & Economics

Knowledge Management Branch | Ministry of Environment & Climate Change Strategy

Mailing adress: PO Box 9347 STN PROV GOVT, Victoria, BC V8W 9M1

Physical adress: 525 Superior St, Victoria, BC V8V 1T7

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Fax: 250-356-7197

From: Tabata, Amy [mailto:Amy.Tabata@dfo-mpo.gc.ca]

**Sent:** Monday, December 11, 2017 10:55 AM **To:** Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

We are currently processing the samples, and expect results in the next few days.

Please contact

Dr Kristi Miller – Head – Molecular Genetics Lab, cc'd above or by phone at 250-756-7155

Thanks

## Amy Tabata

Molecular Genetics Technician Fisheries and Oceans, Canada Pacific Biological Station 3190 Hammond Bay Road

s.16(2)(c)

Nanaimo, B.C. V9T 6N7 ph. 250-756-3369 fax 250-756-7031 email amy.tabata@dfo-mpo.gc.ca

From: Tesch, David ENV:EX [mailto:David.Tesch@gov.bc.ca]

Sent: December-11-17 10:35 AM

To: 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy; McRae, Jake (EC)

Subject: RE: PRV Sampling

Thanks Ken,

I've been able to get a hold of Joyce and she is going to give DFO a call.

Regards,

D.

From: Russell, Ken (EC) [mailto:ken.russell@canada.ca]

Sent: Monday, December 11, 2017 10:29 AM

To: Tesch, David ENV:EX

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy (Amy.Tabata@dfo-mpo.gc.ca); McRae, Jake (EC)

Subject: RE: PRV Sampling

Good morning David,

I am not sure of the analysis time line for the PRV. Your best contact at this point in time would be Laura Hunse – who is in contact with Amy Tabata. Ms. Tabata is the DFO molecular geneticist technician who accompanied us during the sampling. I have CCed Ms. Hunse and Ms. Tabata on this Email.

I hope this helps

Ken Russell

Senior Enforcement Officer - Enforcement Branch

Environment and Climate Change Canada / Government of Canada

ken.russell@canada.ca / Tel : 250 756 7251 / Cell :

Ken Russell,

Agent d'application de la loi Supérieur, Direction gènèrale de l'application de la loi

Environement et Changement Climatique / Gouvernement du Canada

ken.russell@canada.ca / Tel.: 250 756 7251 / Tel. Cell:

From: Tesch, David ENV:EX [mailto:David.Tesch@qov.bc.ca]

Sent: December 11, 2017 9:12 AM

To: Russell, Ken (EC)
Cc: Austin, Joyce ENV:EX
Subject: PRV Sampling
Importance: High

s.16(2)(c)

Hi Ken,

My name is David Tesch and I am Joyce's Executive Director. Joyce is not in the office today and my DM is asking if there is an ETA on the results from the PRV sampling that was done at the fish farms early last week. Are you able to provide me an answer?

Regards,
David Tesch
Executive Director
Knowledge Management Branch
Ministry of Environment & Climate Change Strategy
778-698-4406
David.Tesch@gov.bc.ca

Kristi Miller, PhD
Emiliano Di Cicco DVM PhD
Fish Health Researcher
PSF - DFO







## **HSMI in Atlantic salmon:**

- Worldwide, HSMI outbreaks have always occurred in association with PRV-I; PRV is both statistically and spatially associated with developing lesions
- The cause and effect relationship between PRV-I and HSMI was definitively established in Norway in 2017 (Rimstad's talk)
- Genome sequencing shows that the strain of PRV in BC salmon is >97% identity with that causing HSMI in Norway (PRV-Ia)
- lesions in >80% of the farm population, and showing the same linkage with HSMI was recently reported on a salmon farm in BC, with inflammatory PRV as observed in other farm outbreaks
- HSMI lesions have also been observed in farm audit samples, suggesting that the disease is not limited to this one farm.
- Norway...especially given the high identity of BC and Norwegian agents If PRV causes HSMI in Norway, it causes HSMI in BC; not scientifically defensible that another agent would be the cause here than in

disease development pathway and figgers, fransmission and risk to Total to so of the state of the 

## Jaundice in Pacific Salmon:

- have been observed in Norway, Chile, and Japan in association with various Outbreaks of diseases in Pacific salmon characterized by inclusion bodies (EIBS), jaundice, anemia, and often mild, transitory HSMI-like heart lesions strains of PRV
- A cause and effect relationship (using purified virus) has been established between PRV-2 and the disease in Coho salmon
- Norwegian Rainbow Trout, but a cause and effect relationship was not A challenge study with PRV-3 was able to emulate the heart lesions in established (tissue homogenate)
- In Chile, challenge studies have not yet taken place, but outbreaks of disease in Coho salmon have occurred in association with PRV-3 (and possibly PRV-
- In BC, despite extensive sequencing, only one strain of PRV has been found, PRV-la, which causes HSMI in Atlantic salmon
- observed in farmed Coho and Rainbow trout, but in this case, in association However, BC Chinook salmon show the same disease manifestation as with the same strain of PRV that causes HSMI (PRV-I).

broviding critical data for PRV risk assessments to Pacific sallon Research of Chicok salton for audit samples is beginning to elicidate the role of PRVIa in the disease development pathway

## Outline

## Jaundice in Pacific salmon:

- What we can learn from studies around the world
- PRV Prevalence distribution in farmed Chinook salmon
- Spatial variation of PRV among management zones
- Disease Development
- Identifying fish in an early stage of disease development
- Viral disease diagnostic panel application differentiates fish that are PRV carriers vs those with molecular evidence of disease
- n siu hybridization reveals where the virus is localized during the jaundice disease development purposed

## Norwegian Rainbow Trout

Disease characterized as HSMI-like

Temporal:

hatchery and up to 4 months after SW transfer (over 6 months) Clinical: anorexia, lethargy, modest to 21% mortality in FW

Gross: haemorrhages, ascites, anaemia, bulging eyes, jaundice yellow liver

hepatocytes, haemosiderosis in spleen, increased circulatory neutrophils in kidney and spleen **Pathological**: All fish showed pancarditis- *especially spongy layer*, some with cardiomyocyte necrosis, degeneration and necrosis of red muscle fibers, fibrosis, vacuolization and necrosis of

Haematology: reduced haematocrit (22%)

Electron microscopy: no EIBS detected

heathy fish from tanks without disease no PRV; live-sampled fish from tanks with disease also had PRV-3 (Om) strain sequence identified in high load (CT 22) in all affected fish across 5 farms vs. high load detections suggesting that like HSMI, disease may occur farm-wide

Suggested next steps: controlled challenge studies, reveal pathogenesis and assess tissue distribution of PRV at different stages of infection Olsen et al. 2015. PloS one, 10(7), p.e0131638.

## Norwegian Rainbow Trout

PRV-3 (0m)

IP injection/cohab trial with tissue homogenate showed:

Viral replication in red blood cells in both Atlantic salmon (AS) and Rainbow trout (RT)

Histopathology examination limited to the heart

RT: Cohab peak infection at 6 wks(Ct 23, 80-100% infected), with mild to moderate heart lesions 8 WPI in 1/3<sup>rd</sup> of fish, strong antiviral response, inclusion bodies observed, ascites, pale gills and hemorrhages

myocarditis in only a few fish, inclusion bodies, inclusion of hypoxia and crowding stress AS: Cohab infection took 8-16 WPI, <50% infected, weak antiviral response, mild focal increased infection rates, but not disease development, weak anti-viral response PRV-3 behaves more like an acute infection in RT, with viral peak and subsequent clearance, compared to PRV-I infections in AS, which are chronic and long lasting Study concludes that while AS don't appear to be strongly impacted by PRV-Om, they could be a reservoir for infection of RT!

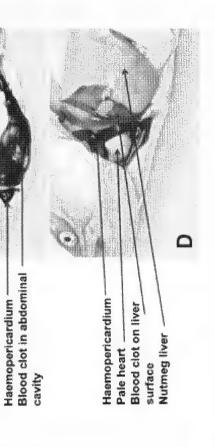
Suggest that "early antiviral response may play and important role in triggering pathology"

Hauge et al. 2017, PloS one, 12(7), p.e0180293

## 01720

## Chilean Coho salmon

Investigation of PRV-related farm outbreak of disease; "HSMI-like" disease in Coho salmon



Targeted sampling: lethargy and morbidity

Gross: jaundice, pale heart, ascites, blood clots in abdominal cavity

muscle, major hepatic necrosis in fish with low Cts, erythrophagocytosis **Pathology**: Myocarditis restricted to spougy layer, minor myositis of red in kidney/spleen

Coho contained a diverse range of PRV genotypes—Ia, Ib, and II No effort to differentiate the strains associated with lesions Godoy et al., 2016, Virology J 13: 98

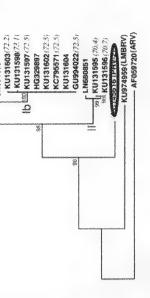
## 01721

## Japanese Coho salmon

KU131593 (78.5) KU131594 (78.5) KU131594 (78.2) KU131591 (78.2) KT456501 (78.2) KR872636

> challenge study—Novel PRV-2 variant identified as the Viral purification from EIBS-affected Coho used in cause of EIBS in Japanese Coho salmon

erythrocytic inclusion bodies, jaundice assumed to be caused by EIBS is traditionally characterized by severe anemia and excess bilirubin in the liver



Consistency in pathological changes in Chilean Coho salmon:

Yellow liver-jaundice, pale gills, splenomegaly, hemopericardium, epicarditis, myocarditis

in naïve fish, but no significant increase in virus and no EIBS in previously exposed establishing Challenge study showed: High loads of virus and inclusion bodies developed post-challenge that protective immunity occurs

Farm Epizootic: PRV-2 peak copy numbers in intestine, kidney, liver, muscle and spleen 1 week earlier than in heart, hematocrit decreased coincident increased with PRV-2 load, cumulative mortality of 23%

Previous observation that EIBS is a higher risk in fish fed large amounts of food for rapid growth Takano et al. 2016, *PloS one*, 11(10), p.e0165424

# Examples of "Yellow" Jaundice fish caught in the wild



Sockeye salmon Tseshaut FN, Port Alberni July 2011



Chinook salmon Newport, Oregon July 2012



Pink salmon Fraser River Fall 2014



Sockeye salmon Copper River, AK August 2008

## 01723

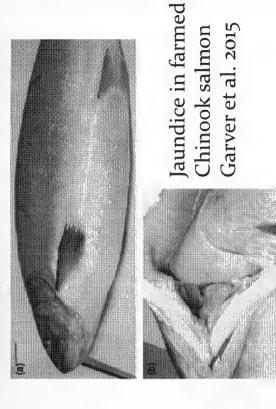
## **BC Chinook Salmon**

Jaundice is described as a recurrent "syndrome" sporadically affecting cultured Chinook salmon

Clinical: off feed

Gross: jaundice, anemia, pale liver

Moderation of the second of th Pathology: Pepatic necrosis, hepatocelular 



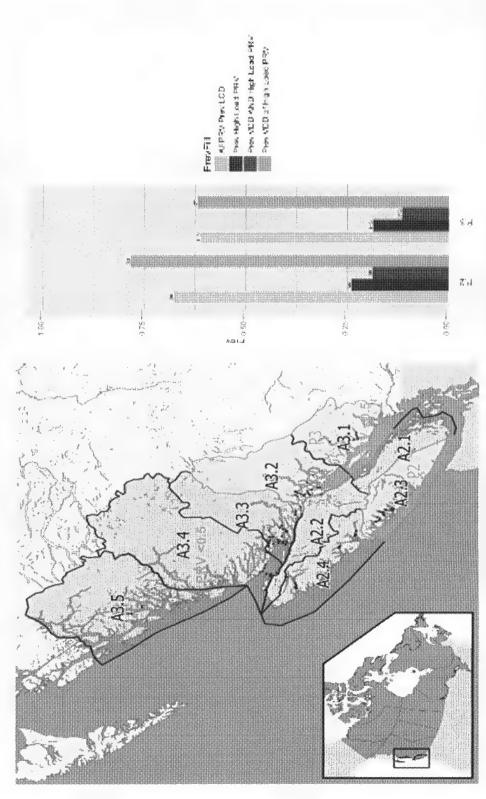
PRV-Ia is the only strain of PRV that has been detected in BC salmon, and all 10 farm-jaundice fish from the Garver study contained high loads of PRV

PRV in relation to Jaundice was actually first detected in my lab in 2011 in a study

21(1)(b)

Garver et al. 2015, J. Fish Dis. Doi:10.1111.jfd.12329

## 93% of Jaundice fish in area P2—West Coast of Vancouver Island



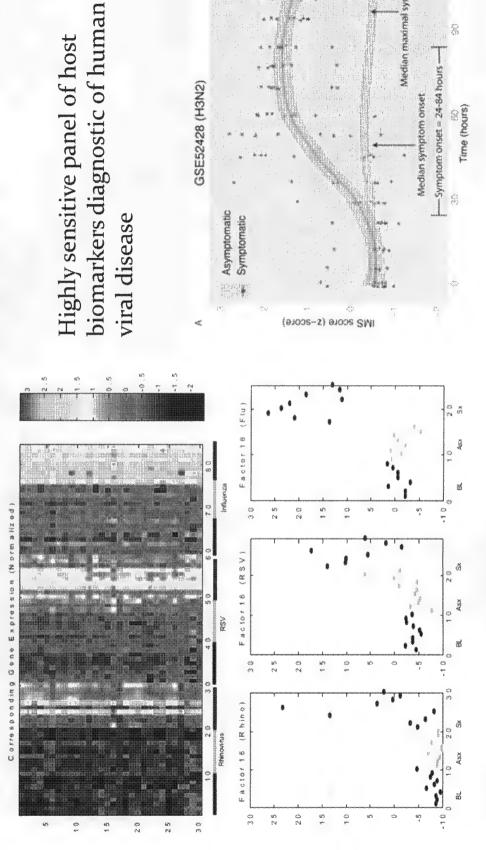
PRV Prevalence Farmed Chinook Salmon

The role of PRV in the development of Jaundice can be substantiated by:

1) employment of a novel, highly sensitive molecular tool to recognize early stages of viral disease development 2) employment of in situ hybridization to localize PRV within tissues of fish developing jaundice

## 001726

# Molecular Viral Disease Diagnostic (VDD) biomarkers in humans



baseline

asymptomatic

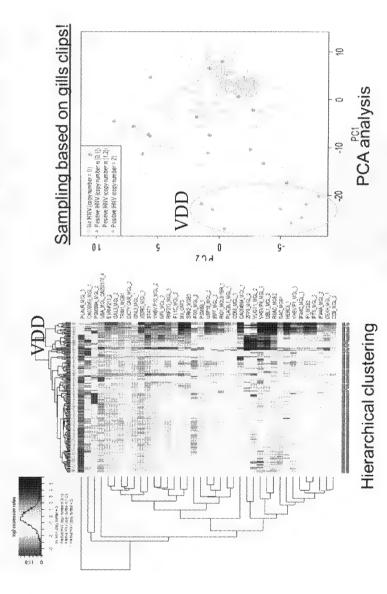
symptomatic

Symptomatic Respiratory Viral Infections in Humans" Cell Host & Microbe 6, 207-217 Zaas et al., 2009 "Gene Expression Signatures Diagnose Influenza and Other

# Molecular Viral Disease Diagnostic (VDD) biomarkers in Salmon

Similar approach taken to develop a panel of viral disease biomarkers for salmon ISAV, PMCV, IPNV, IHNV, PRV, and others..., and a range of tissues predictive of viral disease from emanating from any RNA virus

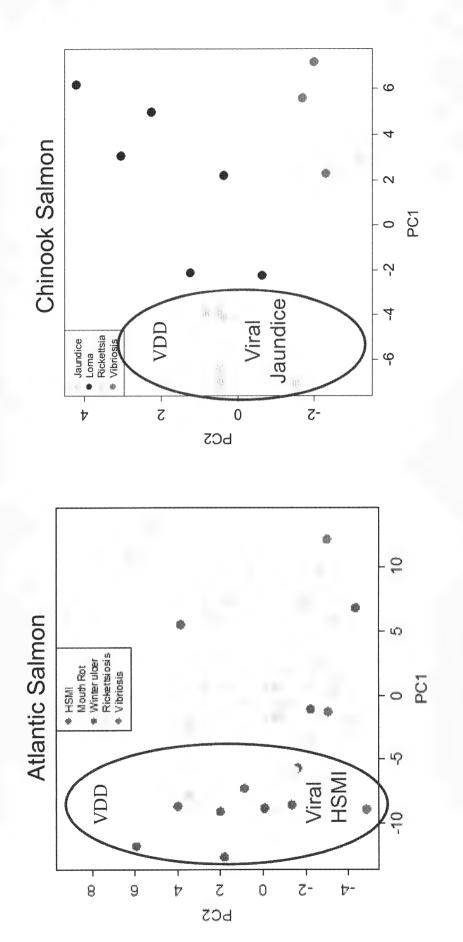
Most wild migrating sockeye salmon smolts with high IHNV loads "VDD"



Miller et al. 2017 Conservation Physiology 5(1)

# Molecular VDD biomarkers – early disease detection

PRV-related viral diseases in both Atlantic and Chinook salmon audits differentiated by VDD



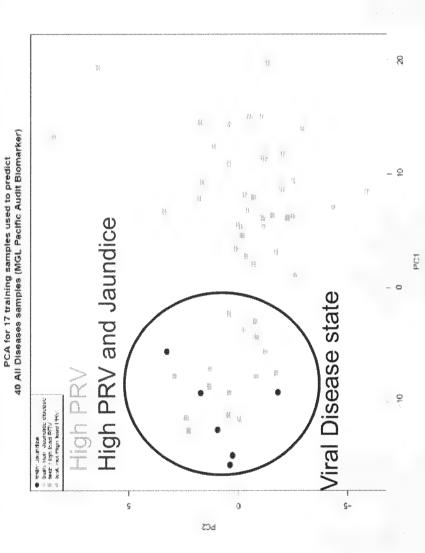
Miller et al. 2017 Conservation Physiology 5(1)

## 001729

## Molecular VDD biomarkers - Piscine Orthoreovirus

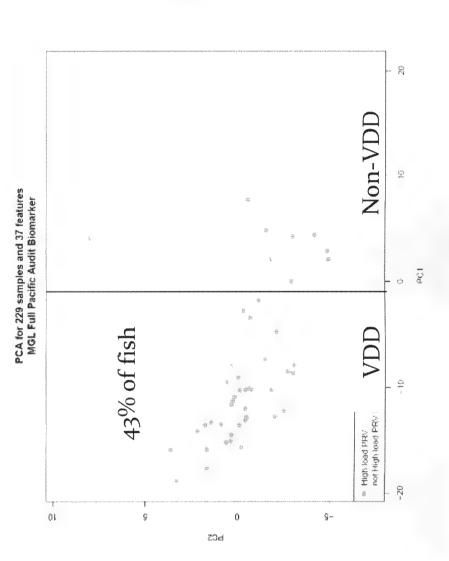
Farmed Chinook salmon audits

80% of farmed Chinook salmon with high loads of PRV are in a "viral disease state" 50% of which were diagnosed with jaundice/anemia



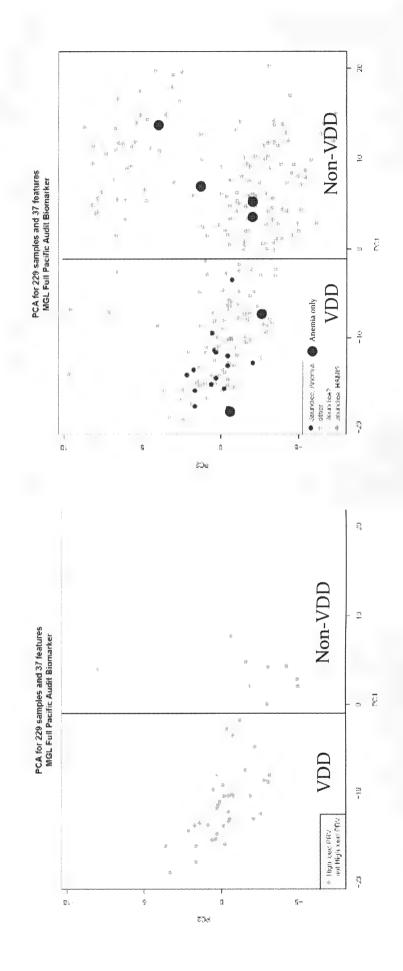
## Molecular VDD biomarkers - Piscine Orthoreovirus Farmed Chinook salmon

43% of dying Chinook salmon are in a VDD state-65% with unknown viral associations



# Association of PRV with Jaundice in Chinook salmon

While many jaundice fish also have anemia, not all fish characterized only with anemia fit this pattern All fish characterized with jaundice carry high loads of PRV, and all are classified as VDD >5% of audit Chinook salmon dying with jaundice, 14% on the west coast alone

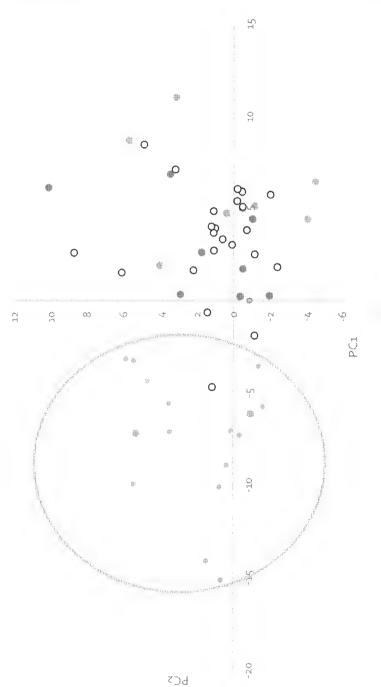


## 001732

## Molecular VDD biomarkers - Piscine Orthoreovirus Wild Chinook Salmon

93% of Wild Chinook juveniles containing high loads of PRV are in a "viral disease state"

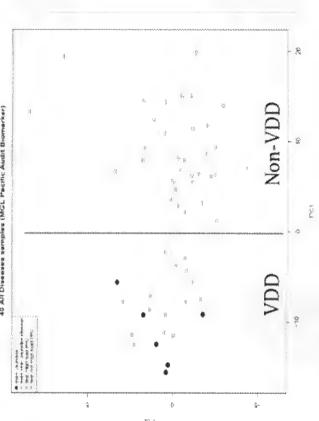




High \* Low \* Moderate o Negative

unique opportunity to study disease development pathways both in laboratory and field applications—given its ability to identify individuals at an early (pre-clinical) stage of disease differentiate viral carriers from active disease states, and to The molecular viral disease diagnostic (VDD) panel offers a development





## In-Situ Hybridization for PRV-Ia

By detecting the localization of PRV in farmed Chinook salmon collected for the DFO - Audit program

we want to better understand the development pathway of Jaundice/Anemia

Jaundice → 1. PRV+

2. PRV+/VDD

3. PRV+/Jaundice

Liver – Kidney – Spleen – Intestine - Heart

PRV+/VD

LIVER PRV (red) in damaged and necrotic hepatocytes (arrowheads

has begun to infect other Tissues—here the hepatocytes, where there is also evidence of In fish classifying as VDD+ there is evidence that PRV in a viral disease state) necrosis

## PRV+/Jaundice LIVER PRV (red) in the damaged hepatocytes

In fish classifying as Jaundice (all also VDD) the virus continues to be associated with expansive areas of liver necrosis

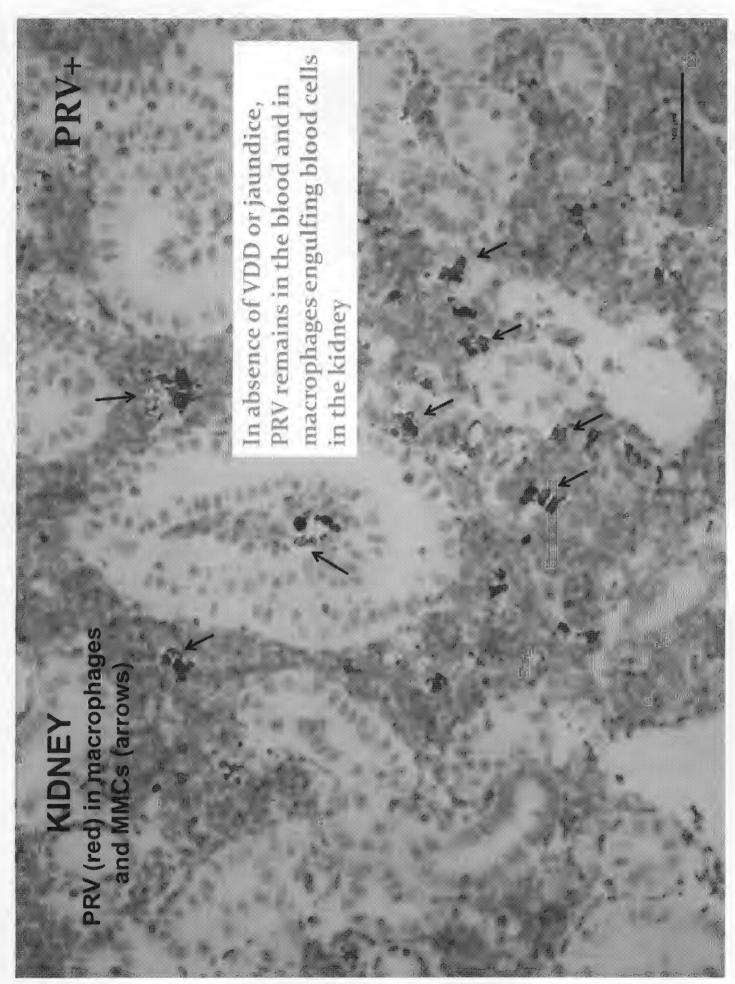
PRV (red) is initially present in the RBCs only, while it moves in hepatocytes in the VDD phase, and localizes in the necrotic lesions as Jaundice develops

PRV+/VDD

PRV+

WDD F

PRV+/Jaundice



#### KIDNEY

PRV (red) in macrophages and MMCs (arrowheads)

PRV+

In absence of VDD or jaundice,
PRV remains in the blood and in
macrophages engulfing blood cell
around the kidney tubules





In fish positive for VDD but not jaundice, PRV continues to be strong in macrophages but also starts infecting kidney tubules which are becoming necrotic (\*)

#### KIDNEY

PRV (red) in macrophages and MMCs (arrowheads), and in the necrotic material inside the renal tubules (arrows)



#### KIDNEY

PRV (red) localizes in the macrophages cells, mostly around the tubules, as well in the epithelial cells of the tubules and in the necrotic material inside the damaged tubules

PRV+/VDD

PRV+/Jaundice

SPLEEN
PRV (red) in the Macrophages,
MMC and RBCs

In absence of VDD or jaundice, PRV remains in the blood running through the spleen

PRV (red) in the Macrophages,
MMC and RBCs in sinuses (arrows)

PRV+/VDD

In fish positive for VDD but not jaundice, there is evidence of massive blood cell lysis in the spleen, releasing large amounts of virus engulfed by macrophages and MMCs

#### SPLEEN

PRV (red) in the Macrophages, MMC and RBCs

PRV+/Jaundice

In fish with jaundice, the spleen carries massive levels of PRV within macrophages and infecting other cells

#### SPLEEN

PRV (red) is initially present in the RBCs, then the macrophages and MMCs start phagocyting the damaged cells, to the point to engulf the organ during the Jaundice phase

PRV+/VDD

PRV+/Jaundice

#### PRV+/VD enterocytes of the intestine. This may be In fish positive for VDD but not jaundice, Virus moves out of RBCs and infects PRV (red) in the RBCs (arrowheads) and enterocytes (arrows) INTESTINE a route of viral exit

## PRV+/Jaundice

PRV (red) in the RBCs (arrowheads)

INTESTINE

and enterocytes (arrows)

In fish with jaundice, PRV continues to infect enterocytes of the intestine. This may be a route of viral exit

### PRV+/Jaundice PRV (red) is initially the RBCs only, but it localizes also in the enterocytes in the VDD and Jaundice phases PRV+/VDD

Compact Myocardium

#### HEART

PRV (red) in RBCs and cardiomyocytes, mostly in the spongy myocardium, and associated to endo/myocarditis

In fish with VDD but not yet jaundice, as in Atlantic salmon, PRV moves out of the RBCs and begins infecting cardiomyocytes

#### HEART

PRV+/VDD

PRV (red) in RBCs and cardiomyocytes, mostly in the spongy myocardium, and associated to endo/myocarditis

Epicardium

begins infecting cardiomyocytes, PRV moves out of the RBCs and jaundice, as in Atlantic salmon, concentrating in areas of mild In fish with VDD but not yet infammation.

Spongy

Myocardium

Compact Myocardium

## HEART (Spongy)

PRV (red) in RBCs

and cardiomyocytes (arrowheads)

PRV+/VDD

In fish with VDD but not yet jaundice, as in Atlantic salmon, PRV moves out of the RBCs and begins infecting cardiomyocytes, concentrating in areas of mild inflammation

PRV+/Jaundice

Compact Myocardium

Epicardium

By the time a Chinook salmon becomes jaundice, heart inflammation has largely dissipated and PRV all but disappears from the heart

#### HEART

PRV (red) in RBCs (arrowheads)
and cardiomyocytes,
mostly in the spongy myocardium (arrows)

Spongy Myocardium

#### 01760

#### HEART

moving into the cardiomyocytes (mostly in the PRV (red) is initially present in the RBCs, but spongy myocardium), particularly in the VDD phase, and receding in the Jaundice phase

PRV+/VDD

PRV+/Jaundice

PRV+

Jaundice (Chinook salmon) Similarities and Differences HSMI (Atlantic salmon) Between and

### HSMI vs Jaundice Spleen

# Summary – HSMI/Jaundice PRV (-/+++) vs Lesions (0-3)

		Liver	Kidney	Spleen	Heart	Intestine	Blood	
u	DRV+/No Lecions		+	+			‡	J
ow		0	0	0	0	0		ior
IBS	DRV+/Neveloping lesions	‡	‡	++++	++	+	++	ew
utic		0	1	2	1 to 2	0		e une
tlaı	DRV/+/HCMI	+	+	++++	+ + +	-	++	IJu
A		1	1	2	3	0		
u	DRV+	ı	+	+	8	ŧ	+	
ow		0	Н	0	0	0		-
es :	PRV+/VND	+ + +	++++	+++	+	+	+++	
ООК		2	3	2	2	0		бто
uıų	PRV+/laundice	‡	++++	+	+	+	++	-
C		3	3	2	0 to 1	0		

Inflammation

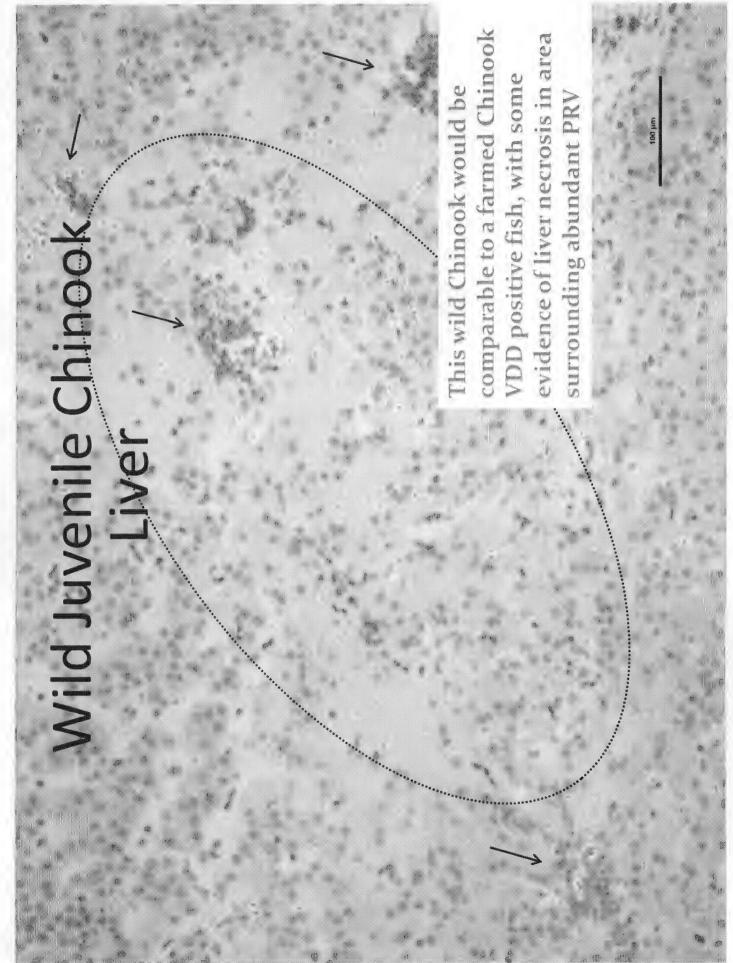
Degeneration

# Wild Juvenile Chinook

# West Coast Vancouver Island

PRV+/VDD

# Wild Juvenile Chinook Liver



### Wild Juvenile Chinook Posterior Kidney

This wild Chinook would be comparable to a farmed Chinook VDD positive fish—but not yet fully necrotic in kidney

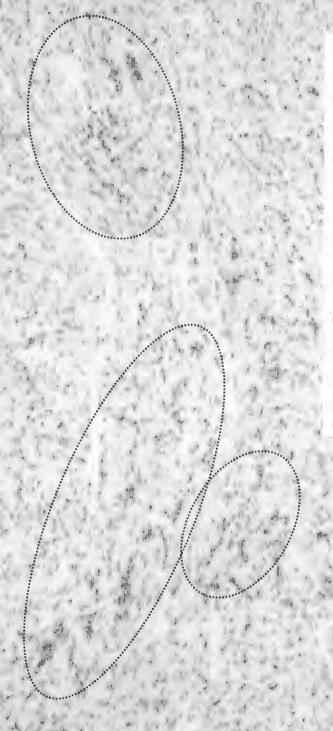
## Wild Juvenile Chinook Spleen

Brownish stain indicates massive RBC lysis, red indicates very large abundance of virus in spleen of wild Chinook salmon—similar to what we see in farmed Chinook

### Wild Juvenile Chinook Heart

Evidence of mild heart lesions in PRV infected wild salmon indicate an infection equivalent to a VDD positive farmed Chinook salmon

## Wild Juvenile Chinool



PRV is infecting areas where heart inflammation is developing

# Wild Juvenile Chinook Intestine

### 01779

## Conclusions

In BC, PRV-Ia is likely causative of diseases that manifest differently in Atlantic and Pacific salmon: HSMI and Jaundice/Anemia

- HSMI is an inflammatory disease affecting heart and skeletal muscle
- Jaundice/anemia is a necrotic disease primarily affecting the liver and kidney, but with transient inflammatory lesions in the heart
- In both diseases, PRV begins by infecting RBCs; there is no evidence of disease or VDD in fish while PRV is exclusively in the blood
- In both diseases, once the virus is released to infect other tissues, a host viral disease (VDD) is stimulated and pathological lesions may develop
- In HSMI, the virus appears to be released without rupturing the red blood cells; in jaundice/anemia, mass lysis of RBCs is evidenced by anemia and hemosiderin, resulting in the release of hemoglobin in levels in excess of what can be processed by hepatocytes and kidney tubules
- While there is evidence that PRV-Ia infects liver and kidney cells that hemoglobin toxicity or viral infection, but in either case, the virus is become necrotic, it is not clear if they become necrotic due to clearly involved

### 01780

## Conclusions

As in Norway, it is highly likely that PRV-related diseases are more prevalent than currently understood

- not currently classified as PRV-related diseases, but many contain lesions VDD panel identifies fish at early stages of disease development that are that are in the developmental pathway towards the full disease
- diagnosed non-viral diseases as the cause of death, but may also contain PRV may be playing a co-infection role in the manifestation of other diseases; many fish with high loads of PRV classifying as VDD are lesions associated with HSMI or jaundice

carefully when determining the level of risk that high concentrations of this virus Our evidence shows the same strain of PRV (Ia) has a role in diseases developing in both Atlantic and Chinook salmon, information that should be considered in farmed salmon pose to wild salmon in BC.

While not yet demonstrating cause and effect (with jaundice), the localization of the virus in tissues clearly demonstrates the virus has a role in disease development in Chinook salmon

### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

December-11-17 9:42 PM

To:

Brian Riddell

Subject:

RE: PRV In Situ Paper Outline.docx

Well if we removed the sequencing and vdd for sshi farmed fish we might be able to push it out faster. I will talk to my team.

From: Brian Riddell [briddell@PSF.CA] Sent: December 11, 2017 9:14 PM

To: Miller-Saunders, Kristi

Subject: RE: PRV In Situ Paper Outline.docx

My only concern is the Provincial Panel will be delivered in the beginning of February and they will be looking for hard advice. If we can get a manuscript organized in that timeframe that would be great but then we will have to contend with the communication plan and approval within DFO.

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

Sent: December 11, 2017 9:05 PM To: Brian Riddell <a href="mailto:sriddell@PSF.CA">briddell@PSF.CA</a>

Subject: FW: PRV In Situ Paper Outline.docx

In answer to your question, yes we have been talking. See outline above. It will take an extra month or so, but I would really like to see a comparative piece on HSMI and Jaundice, showing the in situ localization of the virus in both diseases. I think when you understand that really the key difference between the two diseases is really just the massive lysis of red blood cells in Jaundice versus trickling out of virus in HSMI, the fact that there can be two superficially differently appearing diseases in two species makes more sense and would provide more powerful evidence of a linkage between PRV and disease development.

Kristi

From: Miller-Saunders, Kristi

Sent: December 11, 2017 11:43 AM

To: DiCicco, Emiliano

Subject: PRV In Situ Paper Outline.docx

This would be my ideal paper, not just a note, but something definitive that would show that the SAME virus likely causes TWO related but different diseases in Atlantic and Pacific Salmon. We can discuss other options, but this could be very powerful. Ideally we would follow up with a paper collaborating with Chile, Norway, and Japan and doing in situ hybridization on their Pacific salmon species with PRV-related diseases using both our probe and probes designed to their strains... We should start contacting them with this possibility sooner rather than later. For our initial paper, it would be useful to at least get a sample from Norwegian RT to work with from Espen or Oystein so that we can copublish with them if they are interested.

Kristi

### Miller-Saunders, Kristi

Nathan

From: Sent: To: Subject:	Taylor, Nathan December-13-17 6:02 AM Miller-Saunders, Kristi	
FYI		
From: Townsend, Jill Sent: Tuesday, December 12, 20 To: Taylor, Nathan Subject:	017 4:40 PM	we fin experience a version
From: Taylor, Nathan Sent: December-12-17 2:51 PM To: Townsend, Jill Subject:		gan Paraga . The flag the relative flower
From: Townsend, Jill Sent: Tuesday, December 05, 20 To: Taylor, Nathan Cc: Lowe, Carmel Subject:		s.14(a) s.19(1) s.21(1)(a
Thanks Nathan,		s.21(1)(b s.23
Jill From: Taylor, Nathan Sent: December-05-17 3:46 PM To: Townsend, Jill Cc: Lowe, Carmel Subject:		rha sair sair sairtean an
Best		

From: Townsend, Jill Sent: Tuesday, December 05, 2017 12:03 PM To: Taylor, Nathan Cc: Lowe, Carmel Subject:	
Hello Nathan,	
Jill Townsend Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches et Océans Canada, Pacific Regional Office   Région du Pacifiq 401 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, Jill.Townsend@dfo-mpo.gc.ca Telephone   Téléphone (604) 658-Cell   Government of Canada   Gouvernement du Canada   **SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNSEL): This email has been prepared at the request counsel and the contents of this email are subject to solicitor client privilege. Any use, disclosure or copying of the information strictly prohibited.**	-2843, of
Strictly promoted.	s.14(a) s.16(2)(c)
From: Taylor, Nathan	s.19(1)
Sent: December-05-17 9:13 AM  To: Townsend, Jill  Cc: Lowe, Carmel  Subject:	s.21(1)(a) s.21(1)(b) s.23
HI JIII.	
N.	

### Page 1784 is withheld pursuant to sections est retenue en vertu des articles

14(a), 21(1)(b), 23, 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-13-17 9:02 AM

To:

Parsons, Jay

Subject:

FW: Aquaculture testing

**Categories:** 

ATIP

fyi

From: McPherson, Arran

Sent: December 12, 2017 5:24 PM

To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca >; Moore, Wayne < Wayne.Moore@dfo-mpo.gc.ca >

Subject: FW: Aquaculture testing

FYI.

From: McPherson, Arran

Sent: Tuesday, December 12, 2017 5:24 PM

To: Blewett, Catherine < Catherine. Blewett@dfo-mpo.gc.ca>

Cc: Reid, Rebecca < Rebecca.Reid@dfo-mpo.gc.ca>; Hopkins, Lillian < Lillian.Hopkins@dfo-mpo.gc.ca>; White, Andrea

<<u>Andrea.White@dfo-mpo.gc.ca</u>> **Subject:** Aquaculture testing

Catherine, just a quick head's up that DFO Science is assisting ECCC/Province's efforts related to their investigations into recent effluent discharged at Tofino and Brown's Bay aquaculture sites.

DFO Science assisted in sample collection on Dec 4-6<sup>th</sup> and in response to a request, will be processing PRV testing this week. While this work is underway, it has been clarified that requests for advice from ECCC/province should go through the CSAS process to ensure the right experts are involved, peer review takes place (and for tracking purposes).

We will also be connecting with Province of BC to make them aware of this process as well. We will keep you advised on outcome of the results/release. I suggest this be shared with MINO for their info re our involvement.

Arran.

### Dickie, Catherine

From:

Lowe, Carmel

Sent:

December 13, 2017 10:06 AM

To:

Miller-Saunders, Kristi; Taylor, Nathan

Cc:

MacDougall, Lesley

Subject:

PRV testing

Both – thanks for letting me know your schedules. Lesley and I met and she is going to start populating a Rapid Science Response with the (minimal) information I have been able to provide to her. It will require your input for sure. It is not a particularly onerous template – just needs to capture what has been requested, by whom, for what. What we have done to respond and how. I am thinking we would simply attach the analytical results as an 'Annex' to the response.

Suggest we meet tomorrow afternoon to ensure we are all clear on whose doing what in what timeframes.... Etc.

### Carmel

Carmel Lowe, Ph.D.
Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.gc.ca
Telephone | Téléphone 250-756-7177
Facsimile | Télécopieur 250-729-8360
Government of Canada | Gouvernement du Canada

### Miller-Saunders, Kristi

From:

Lowe, Carmel

Sent:

December-13-17 5:14 PM

To:

MacDougall, Lesley; Miller-Saunders, Kristi; Taylor, Nathan

Subject:

RE: PRV Sampling

Thanks Lesley. I expect Kristi and Nathan will help with completing/revising the draft – but would like to get an understanding of when they might be available to do so as I further understand that the testing will be completed tomorrow sometime?

Just so everyone is clear on the process the information will be transmitted to clients once the Rapid Science Response is approved. I am in Vancouver for meetings in am but will be back in the office in the aft and happy to review then.

### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

### Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360 Government of Canada | Gouvernement du Canada

From: MacDougall, Lesley

Sent: Wednesday, December 13, 2017 2:09 PM

To: Miller-Saunders, Kristi < Kristi.Saunders@dfo-mpo.gc.ca>; Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>

Cc: Taylor, Nathan < Nathan. Taylor@dfo-mpo.gc.ca>

Subject: RE: PRV Sampling

### Hello all;

I've attached a draft Rapid Science Response, with the preliminary information I've received, as a start to ensure that the results and information resulting from this request can be documented.

As noted - definitely draft, needs input from ADGT

From: Miller-Saunders, Kristi Sent: 2017–December-12 1:24 PM

To: Lowe, Carmel

Cc: Taylor, Nathan; MacDougall, Lesley

Subject: RE: PRV Sampling

This request came to me on a Thursday afternoon, and while I would normally have informed Nathan, he was away. I was not aware of any process involving Lesley MacDougall. They had approached a number of diagnostic labs already, and none of them had experience with water samples. My lab does. Hence, by the time they came to me, they were quite desperate as they had planned on collecting the samples the following Monday-Tuesday and needed to know how they would do this. I involved Curtis Suttle's lab in this conversation as he is a marine virologist with vast experience in isolating viruses from the ocean, and is working with us on this end in the SSHI. There

was no way I would have had time to fill out extensive paperwork on this so I suggested we go ahead and collect the samples, with my technician Amy Tabata's involvement, to ensure they were collected in a way that was useful, and that we had to leave the rest until I returned.

I now know that there is a process and will inform the three of you if any requests of this nature arise in future. I will ask them the question you pose below and let you know how they respond.

Kristi

From: Lowe, Carmel

**Sent:** December-12-17 12:57 PM **To:** Miller-Saunders. Kristi

Cc: Taylor, Nathan; MacDougall, Lesley

Subject: RE: PRV Sampling

Kristi.

Thanks for sharing this with me. I have to say I am a bit concerned at how these requests for sampling and analyses from the province-ECCC were routed directly to you given that our department has formalized approaches/policies for requesting/providing science information and advice to clients. To ensure we are complying with the policies, I would ask that any further requests you receive be directed to Lesley MacDougall in our CSAS office (this should certainly be done for the analyses that are being run now in your lab). Lesley has overall responsibility for managing client requests, including, tracking and reporting, securing review and approval as appropriate and transmitting results to the clients. In case you were not aware, CSAS has also recently developed tools to support the provision of information/advice required under very short timeframes which I suspect may be the case with the requests you have received? Certainly understanding how they propose to use the results in their aquaculture review or otherwise will be important for us to document/understand as would knowing if we can anticipate any additional requests for support in near future. To this end, I would appreciate you getting in touch with your contacts to clarify these elements soonest and sharing the outcome of those discussions with Lesley, Nathan and I. If they do intend using the information-advice you provide in their review then a copy of their TOR for this review would be very helpful.

### Cormel

Carmel Lowe, Ph.D.
Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

### Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360 Government of Canada | Gouvernement du Canada

From: Miller-Saunders, Kristi

**Sent:** Monday, December 11, 2017 9:10 PM **To:** Lowe, Carmel < <u>Carmel.Lowe@dfo-mpo.gc.ca</u>>

Subject: FW: PRV Sampling

Here is what I sent to the BC Ministry of the Environment. Before we release the PRV testing results, I will have a conversation with them about how they might use this information in their aquaculture review and whether DFO can be of any further assistance, as requested.

### Kristi

**From:** Miller-Saunders, Kristi **Sent:** December 11, 2017 8:47 PM

To: Austin, Joyce ENV:EX; Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC)

Subject: RE: PRV Sampling

Joyce,

Enclosed is the talk I gave at a BC Salmon Farmers Association meeting on PRV-HSMI state of knowledge workshop December 4th- 5th attended by Norwegian scientists, industry vets and leaders, BC and US Scientists, and DFO regulators. The talk outlines the most recent research out of my lab on PRV and linkages with disease in Pacific salmon. I apologize that it is long and pretty scientific, but they key points are:

We have demonstrated that PRV infections in Chinook salmon can induce a host response that we have shown previously to be diagnostic of the presence of viral disease. This work was published in Conservation Physiology this year.

We demonstrate that 14% of moribund/dead farmed Chinook salmon on the west coast obtained though the DFO audit program were diagnosed with jaundice/anemia, a disease that around the world has been associated with various strains of PRV. There is only a single strain of PRV in BC, that which is known to cause HSMI in Atlantic salmon. We published on HSMI in BC farmed Atlantic salmon in Feb. 2017 in PlosOne.

We show that throughout the developmental pathway of jaundice and across multiple affected tissues, PRV is localized within the regions and cells that become diseased, whether disease is through cell death (necrosis) in liver and kidney or inflammation in heart. We gave a similar talk on HSMI in Atlantic salmon and also demonstrated PRV localized with inflammatory lesions in heart and skeletal muscle tissue.

The primary infective tissue for PRV in both species is the red blood cells (which is why blood water from farmed fish is potentially a strong risk for PRV transmission to wild fish). We show that while PRV remains exclusively in the blood, even at high levels, it is tolerated and there is no disease response in the host. When the virus leaves the blood cells to infect other tissues/cells, it induces a disease response in the host.

The difference between HSMI in Atlantic salmon and jaundice/anemia in Chinook salmon is that in HSMI, PRV appears to leave the red blood cells without lysing (rupturing) them, whereas in Chinook salmon, there is massive lysis of red blood cells leading to anemia (pale gills and tissues) and overloading the kidney and liver with Heme from the breakdown of hemoglobin. Heme is processed in kidney and liver, but becomes toxic at high levels, leading to necrosis (death) of kidney tubules and hematocytes (liver cells), and a jaundice (yellowing) appearance in the fish. While we show that the virus also directly infects these cells, we suspect the heme overload, caused by PRV lysis of red blood cells, is likely the main mechanism leading to disease in jaundice fish. Liver and kidney are not highly affected in HSMI in Atlantic salmon, as the virus goes on to infect muscle cells (heart and skeletal) causing inflammation. This inflammatory response is present, but much reduced in Chinook salmon with jaundice.

We have also demonstrated early (jaundice) disease development in wild Chinook salmon. There was a presentation by Dr. Maureen Purcell at the same meeting that showed an association of the same strain of PRV with a similar disease, which they and the Japanese call EIBS, in Washington State Coho salmon.

I hope this helps in your consideration of the potential for risk in the release of blood water. I am happy to discuss these results directly if there is any need for clarification. We are working up a publication on these data at present.

Kristi Miller-Saunders Head, Molecular Genetics Pacific Biological Station

From: Austin, Joyce ENV:EX [Joyce.Austin@gov.bc.ca]

**Sent:** December 11, 2017 10:58 AM

To: Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

Hi Amy,

I have already contacted Kristi Miller and I'm waiting on her to give me a call back,

Thanks

### Joyce Austin, Ph.D.

Senior Provincial Laboratory Specialist (Unit Head)

**Environmental Monitoring, Reporting & Economics** 

Knowledge Management Branch | Ministry of Environment & Climate Change Strategy

Mailing adress: PO Box <u>9347 STN PROV GOVT</u>, <u>Victoria, BC V8W 9M1</u>

Physical adress: 525 Superior St, Victoria, BC V8V 1T7

Tel.: 778-698-4434;

Cel.:

Fax: 250-356-7197

From: Tabata, Amy [mailto:Amy.Tabata@dfo-mpo.gc.ca]

**Sent:** Monday, December 11, 2017 10:55 AM **To:** Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

We are currently processing the samples, and expect results in the next few days.

### Please contact

Dr Kristi Miller – Head – Molecular Genetics Lab, cc'd above or by phone at 250-756-7155

**Thanks** 

### Amy Tabata

Molecular Genetics Technician Fisheries and Oceans, Canada Pacific Biological Station 3190 Hammond Bay Road Nanaimo, B.C. V9T 6N7 ph. 250-756-3369 fax 250-756-7031 email amy.tabata@dfo-mpo.gc.ca

From: Tesch, David ENV:EX [mailto:David.Tesch@gov.bc.ca]

**Sent:** December-11-17 10:35 AM

To: 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy; McRae, Jake (EC)

Subject: RE: PRV Sampling

Thanks Ken,

s.16(2)(c)

I've been able to get a hold of Joyce and she is going to give DFO a call.

Regards,

D.

From: Russell, Ken (EC) [mailto:ken.russell@canada.ca]

Sent: Monday, December 11, 2017 10:29 AM

To: Tesch, David ENV:EX

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy (Amy.Tabata@dfo-mpo.gc.ca); McRae, Jake (EC)

Subject: RE: PRV Sampling

Good morning David.

I am not sure of the analysis time line for the PRV. Your best contact at this point in time would be Laura Hunse – who is in contact with Amy Tabata. Ms. Tabata is the DFO molecular geneticist technician who accompanied us during the sampling. I have CCed Ms. Hunse and Ms. Tabata on this Email.

I hope this helps

Ken Russell

Senior Enforcement Officer – Enforcement Branch

Environment and Climate Change Canada / Government of Canada

ken.russell@canada.ca / Tel: 250 756 7251 / Cell:

Ken Russell.

Agent d'application de la loi Supérieur, Direction gènèrale de l'application de la loi

Environement et Changement Climatique / Gouvernement du Canada

ken.russell@canada.ca / Tel.: 250 756 7251 / Tel. Cell:

From: Tesch, David ENV:EX [mailto:David.Tesch@gov.bc.ca]

Sent: December 11, 2017 9:12 AM

To: Russell, Ken (EC)
Cc: Austin, Joyce ENV:EX
Subject: PRV Sampling
Importance: High

Hi Ken,

My name is David Tesch and I am Joyce's Executive Director. Joyce is not in the office today and my DM is asking if there is an ETA on the results from the PRV sampling that was done at the fish farms early last week. Are you able to provide me an answer?

Regards,
David Tesch
Executive Director
Knowledge Management Branch
Ministry of Environment & Climate Change Strategy
778-698-4406
David.Tesch@gov.bc.ca

s.16(2)(c)

### Miller-Saunders, Kristi

From:

Miller-Saunders, Kristi

Sent:

December-14-17 9:47 AM

To:

MacDougall, Lesley; Lowe, Carmel

Cc:

Taylor, Nathan

Subject:

RE: Testing fish process effluent

These look good.

Kristi

From: MacDougall, Lesley

Sent: December 14, 2017 9:33 AM

To: Lowe, Carmel

Cc: Taylor, Nathan; Miller-Saunders, Kristi Subject: RE: Testing fish process effluent

HI Carmel; I've provided short answers to the questions posed by DFO Comms below, based on emails and my conversation with Amy yesterday - ADGT folks should probably edit for clarity / accuracy.

How did we come to be involved in the testing?

BC Ministry of Environment & Climate Change Strategies (ENV), has an upcoming compliance audit for two fish processing plants that are currently the focus of investigation. DFO was contacted by ENV to assist with on-site sample collection, and to provide lab analysis of the samples. Other labs available at the time did not have experience with diagnostics from water samples.

Are we working with partners, if so, who?

There is no formal partnership; however, Dr. Curtis Suttle's lab is aware of the lab analyses DFO is currently undertaking. Dr. Suttle is a marine virologist with experience in isolating viruses from marine water samples, and has collaborated with DFO scientists on other marine virology research.

What lab are we using?

The DFO Forensics laboratory, at the Pacific Biological Station in Nanaimo BC, is being used to conduct the sample analyses. The DFO Forensics laboratory is a secure, restricted-access and restricted-use laboratory, where strict protocols are followed to ensure sample integrity.

What pathogens/diseases are we testing for?

DFO has been asked to analyze effluent samples collected from two fish processing plants on December 4 and 5, 2017, for the presence or absence of the Piscine Reovirus (PRV).

When will results be in?

Results will be in by the end of the week (Dec 15)

• What will happen if results show reason for concern / next steps?

Results will be considered in the context of background PRV levels. Further steps may include providing recommendations for marine effluent discharge viral load levels, and potential management and mitigation measures that can reduce risk to the environment (including, for example, recommended effluent treatment options, or proxy testing parameters). These recommendations may be used to guide policy, compliance and enforcement measures.

### Dickie, Catherine

From:

MacDougall, Lesley

Sent:

December 14, 2017 11:03 AM

To:

Miller-Saunders, Kristi; Taylor, Nathan; Lowe, Carmel

Subject:

revised RSR for review prior to discussion today

Attachments:

RSR2017\_AQU01\_Browns and TofinoPRV (2).docx

### Hello all;

Based on emails, discussion with Amy, and the SR we completed on PRV a couple years ago, I've tried to flesh out the request, the process, and the context in advance of receiving the results. All of this will require further edits and input from ADGT staff.

1

Lesley MacDougall BSc, MMM, RPBio

Science Coordinator | Gestionnaire scientifiques

Centre for Science Advice | Centre des avis scientifiques Pacific Region | Région du Pacifique Pacific Biological Station | Station biologique du pacifique Nanaimo, B.C. V9T 6N7 | Nanaimo (CB) V9T 6N7

PHONE #: 250-756-7088 lesley.macdougall@dfo-mpo.qc.ca

### Centre for Science Advice Pacific

FPP non-CSAS Request for Rapid Science Response

	REQUESTINFORMATION			
Request Contact:	Operation of the control of the cont	Project Type: Aquaculture-Emergency response		
Date of request:	December XA, 20 [7	Project footprint:		
Region of proposed impact:	Tofino and Browns Bay, British Columbia	Habitat Type: Coastal		
Purpose of request:	Information for Ministry of Environment / Er	nvironment and Climate Change Canada investigation		
Potential affected species:	Pacific salmon			
Date required:	Oscesby XR, 2017	Request #:2017AQU01		
Timeline rationale:	4 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9			

### **PROJECT OVERVIEW**

As a function of normally-operating fish processing plants, fish waste effluent is released back into the marine ecosystem.

BC Ministry of Environment & Climate Change Strategies (ENV) has an upcoming compliance audit for two fish processing plants that are currently the focus of investigation. The Browns Bay and Tofino fish processing plants have been subjects of recent public and media attention since a video showing fish waste effluent being released into the marine environment was circulated publicly.

DFO was contacted by ENV to assist with on-site sample collection, and to provide lab analysis of the samples. Other labs available at the time did not have experience with diagnostics from water samples.

### 1<sup>57</sup> QUESTION

### Context:

The BC Ministry of Environment & Climate Change Strategies (ENV), Environmental Protection Regional Operations Branch to requested assistance from DFO to advise on appropriate sampling collection methodology, and to test collected effluent for the presence of the Piscine Reovirus (PRV). At the Browns Bay site, effluent was collected from one source – at the plant itself, immediately prior to discharge out of the facility into the environment. At the Tofino site, effluent was collected from two sources; 1) from the fish harvest vessel and 2) from the plant, immediately prior to discharge out of the facility into the environment.

### Objective/Question:

Specifically, ENV is requesting DFO provide advice/expertise to:

- Analyze effluent samples collected from two fish processing plants on December 4 and 5, 2017. EPD may
  require further effluent analyses as part of an upcoming compliance audit of fish processing plants in B.C.
- Assess the PVR data results of presence/absence and if present, to determine whether to test for live virus
  infectivity. From the information and scientific literature you provided, we understand that virus presence in
  effluent to the marine environment is a risk to wild salmon.
- Provide recommendations for marine effluent discharge viral load levels; if the virus levels are a problem, what can be done to minimize impacts and protect the environment. What kind of effluent treatment is recommended for marine discharges? Are there proxy parameters to test?
- Provide information to guide provincial government policy with respect to public health and safety as well as
  protection of the environment, which could include compliance and enforcement.

Importance:	☐ Important	☐ Desirable	
	SCIENCE	RESPONSE	

### Response:

Results from the PCR testing will confirm whether the fish being processed at each of the facilities at that time were infected with PRV; the results will not be able to provide advice regarding the load concentration.

Using the DFO Forensics laboratory, Polymerase Chain Reaction (PCR) testing of the effluent samples from Brown's Bay and Tofino was conducted, to test for the presence of PRV.

Piscine reovirus (PRV) is a non-enveloped, double stranded RNA virus, which is a member of the family Reoviridae (Palacios et al. 2010; Kibenge et al. 2013). PRV was first recognized in Norway, and has since been detected in salmonid and non-salmonid fish in the United Kingdom, Ireland, Denmark, Chile, the United States, and Canada. PRV occurs in populations of wild and farmed salmonids in British Columbia and in wild salmonids in US waters (Alaska and Washington State). However, information with respect to spatial and temporal occurrence of PRV in wild and farmed salmon populations and non-salmonid finfish is limited. This includes knowledge of prevalence of PRV in hatchery stocks in British Columbia. The ubiquitous nature of Piscine Reovirus (PRV), its apparent long time presence in wild Pacific salmonid stocks, and the lack of clear association with disease in laboratory challenge trials, suggest a low likelihood that the presence of this virus in any life stage of farmed Atlantic and Pacific Salmon would have a significant impact on wild Pacific Salmon populations.

While there is general scientific agreement that PRV is typically found in association with Heart and Skeletal Muscle Inflammation (HSMI), understanding the role of PRV in the development of HSMI has been complicated by a lack of culture techniques for this virus. Currently, there is no evidence from laboratory studies in British Columbia and Washington State that PRV infection is associated any disease state, including HSMI. HSMI has not been reported on BC salmon farms.

HSMI was first described and identified as an infectious disease by Kongtorp et al. (Kongtorp et al. 2004; Kongtorp, Taksdal, and Lyngoy 2004). Although several types of viral particles were visualized in HSMI lesions by electron microscopy and a viral etiology was suspected, it was not until 2010 that PRV was identified to be associated with HSMI and a molecular diagnostic test for PRV developed (Palacios et al. 2010; Watanabe et al. 2006). HSMI is one of several diseases that affect the heart and in moderate to severe cases the skeletal muscle of Atlantic Salmon (Biering and Garseth 2012; Kongtorp, Taksdal, and Lyngoy 2004). HSMI cannot be definitively diagnosed by histopathology, unless the affected fish on the farm also have clinical signs consistent with HSMI. Histopathology is used to confirm the diagnosis of HSMI.

PRV is also found in a high proportion of clinically healthy, wild and farmed Atlantic Salmon collected from fresh and saltwater in Norway (Palacios et al. 2010; Lovoll et al. 2010; Garseth et al. 2013). In some instances, PRV loads in wild Atlantic Salmon spawners lacking HSMI were higher than those reported from farmed Atlantic Salmon with HSMI, suggesting that factors other than high PRV loads may be required for HSMI development in the farmed fish (Garseth et al. 2013) (see also the 2014 downloadable report available at the Norwegian Veterinary Institute, Fish Health Reports). The range of PRV loads in farmed fish with HSMI often overlap with those in fish without HSMI, and PRV occurred at similar loads in cohorts of pre-smolts that remained disease free, as compared to cohorts that developed HSMI (Lovoll et al. 2012).

The DFO Forensics laboratory is a limited-access, restricted-use, secure lab where strict protocols are followed to ensure....

...no other samples are processed in the DFO forensics laboratory when a secure sample is being tested....

Results from the PCR testing conclude that...

Browns Bay – processing facility discharge

Tofino – vessel discharge

Tofino – processing facility discharge

Responde	r: XXXX, Science	Responder:
		REVIEWINFORMATION
This response do requirement for S	oes not constitute delivery of Science input.	peer – reviewed Science advice; it is intended as a rapid response to an immediate
Reviewed by: Le	sley MacDougall, Coordinat	or, Centre for Science Advice Pacific Region
Date: X	XXX, 2017	
Comments:		
Approved by:		
Date:		
Comments:		

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-14-17 5:16 PM

To:

Parsons, Jay

Subject:

Re: PRV Sampling

Categories:

**ATIP** 

Ok

Sent from my BlackBerry 10 smartphone on the Rogers network.

Original Message From: Parsons, Jay

Sent: Thursday, December 14, 2017 5:13 PM

To: Moore, Wayne

Subject: Re: PRV Sampling

We are working on it and will be able to get it to you later tonight.

---- Original Message -----From: Moore, Wayne

Sent: Thursday, December 14, 2017 02:06 PM

To: Parsons, Jay

Subject: RE: PRV Sampling

Yeah and there are stacks of others as well. Will bring you up to speed on it when you get back. Do we have the summary of Morton's paper.

----Original Message----

From: Parsons, Jay

Sent: December 14, 2017 4:08 PM

To: Moore, Wayne < Wayne. Moore@dfo-mpo.gc.ca>

Subject: FW: PRV Sampling

Importance: High

fyi

----Original Message-----From: Taylor, Nathan

Sent: December-14-17 3:55 PM

To: Parsons, Jay

Subject: Fw: PRV Sampling

Importance: High

Hey Jay,

Further to your earlier inquiry see below for the most complete response I can provide right now. We'll respond the request (within the domain where we're qualified) with a CSAS Science response.

Hope that's helpful!

NG

---- Original Message ----From: Miller-Saunders, Kristi

Sent: Thursday, December 14, 2017 08:52 AM

To: Lowe, Carmel; Taylor, Nathan Subject: FW: PRV Sampling

Here is the respond to the question you asked Carmel. My lab is cpntri bring to the first and second bullets but they will need a broader array of expertise for the third and fourth bullets. Our test results should be complete today.

Please forward to Leslie as I am working from my phone. Kristi

From: Austin, Joyce ENV:EX [Joyce.Austin@gov.bc.ca]

Sent: December 13, 2017 11:53 AM

To: Miller-Saunders, Kristi; Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC); Freyman, Liz ENV:EX

Subject: RE: PRV Sampling

Hello Kristi,

Thank you for your email and presentation materials. The BC Ministry of Environment & Climate Change Strategies (ENV), Environmental Protection Regional Operations Branch needs the expertise within DFO to:

- Analyze effluent samples collected from two fish processing plants on December 4 and 5, 2017. EPD may require further effluent analyses as part of an upcoming compliance audit of fish processing plants in B.C.
- Assess the PVR data results of presence/absence and if present, to determine whether to test for live virus infectivity. From the information and scientific literature you provided, we understand that virus presence in effluent to the marine environment is a risk to wild salmon.
- Provide recommendations for marine effluent discharge viral load levels; if the virus levels are a problem, what can be done to minimize impacts and protect the environment. What kind of effluent treatment is recommended for marine discharges? Are there proxy parameters to test?
- Provide information to guide provincial government policy with respect to public health and safety as well as protection of the environment, which could include compliance and enforcement.

This is a high priority for the ENV Minister and receiving the results and interpretation are critical for us. Laura Hunse has been designated the person to receive the results although my group is working together with me to respond to questions that are coming from our executive team.

Please don't hesitate to contact me, Laura or Liz if you require more information.

Regards,

Joyce Austin, Ph.D.

Senior Provincial Laboratory Specialist (Unit Head) Environmental Monitoring, Reporting & Economics Knowledge Management Branch | Ministry of Environment & Climate Change Strategy Mailing adress: PO Box 9347 STN PROV GOVT, Victoria, BC V8W 9M1 Physical adress: 525 Superior St, Victoria, BC V8V 1T7

Tel.: 778-698-4434;

Cel.:

Fax: 250-356-7197<tel:250-387-5757>

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

Sent: Tuesday, December 12, 2017 1:47 PM

To: Austin, Joyce ENV:EX; Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC)

Subject: RE: PRV Sampling

Joyce and David,

I have been requested by our regional director of science, Carmel Lowe, to ask you how you propose to use the results we provide in your aquaculture review or otherwise. This is important for us to document/understand internally, and provide answers to our management hierarchy. She would also like to know whether we can anticipate any additional requests for support in near future.

I should have put your request through a formal channel for advice, which I believe is taking place now.

Thanks,

Kristi Miller

Kristi Miller-Saunders, PhD
Head, Molecular Genetics
Pacific Biological Station
3190 Hammond Bay Rd
Nanaimo BC V9T 6N7
250-756-7155
Kristi.Saunders@dfo-mpo.gc.ca<mailto:Kristi.Saunders@dfo-mpo.gc.ca>

From: Austin, Joyce ENV:EX [mailto:Joyce.Austin@gov.bc.ca]

Sent: December-11-17 10:58 AM

To: Tabata, Amy; Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

s.16(2)(c)

Hi Amy,

I have already contacted Kristi Miller and I'm waiting on her to give me a call back,

### Thanks

Joyce Austin, Ph.D.

Senior Provincial Laboratory Specialist (Unit Head) Environmental Monitoring, Reporting & Economics Knowledge Management Branch | Ministry of Environment & Climate Change Strategy Mailing adress: PO Box 9347 STN PROV GOVT, Victoria, BC V8W 9M1 Physical adress: 525 Superior St, Victoria, BC V8V 1T7

Tel.: 778-698-4434;

Cel.:

Fax: 250-356-7197<tel:250-387-5757>

From: Tabata, Amy [mailto:Amy.Tabata@dfo-mpo.gc.ca]

Sent: Monday, December 11, 2017 10:55 AM To: Tesch, David ENV:EX; 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; McRae, Jake (EC); Miller-Saunders, Kristi

Subject: RE: PRV Sampling

We are currently processing the samples, and expect results in the next few days.

Please contact

Dr Kristi Miller - Head - Molecular Genetics Lab, cc'd above or by phone at 250-756-7155

### Thanks

Amy Tabata
Molecular Genetics Technician
Fisheries and Oceans, Canada
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, B.C. V9T 6N7
ph. 250-756-3369
fax 250-756-7031
email amy.tabata@dfo-mpo.gc.ca<mailto:amy.tabata@dfo-mpo.gc.ca>

From: Tesch, David ENV:EX [mailto:David.Tesch@gov.bc.ca]

Sent: December-11-17 10:35 AM

To: 'Russell, Ken (EC)'

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy; McRae, Jake (EC)

Subject: RE: PRV Sampling

Thanks Ken, s.16(2)(c)

I've been able to get a hold of Joyce and she is going to give DFO a call.

Regards,

D.

From: Russell, Ken (EC) [mailto:ken.russell@canada.ca]

Sent: Monday, December 11, 2017 10:29 AM

To: Tesch, David ENV:EX

Cc: Austin, Joyce ENV:EX; Hunse, Laura A ENV:EX; Tabata, Amy (Amy.Tabata@dfo-mpo.gc.ca<mailto:Amy.Tabata@dfo-

mpo.gc.ca>); McRae, Jake (EC) Subject: RE: PRV Sampling

Good morning David,

I am not sure of the analysis time line for the PRV. Your best contact at this point in time would be Laura Hunse – who is in contact with Amy Tabata. Ms. Tabata is the DFO molecular geneticist technician who accompanied us during the sampling. I have CCed Ms. Hunse and Ms. Tabata on this Email.

I hope this helps

Ken Russell

Senior Enforcement Officer – Enforcement Branch Environment and Climate Change Canada / Government of Canada ken.russell@canada.ca<mailto:ken.russell@canada.ca> / Tel: 250 756 7251 / Cell:

Ken Russell,

Agent d'application de la loi Supérieur, Direction gènèrale de l'application de la loi Environement et Changement Climatique / Gouvernement du Canada ken.russell@canada.ca<mailto:ken.russell@canada.ca> / Tel. : 250 756 7251 / Tel. Cell :

From: Tesch, David ENV:EX [mailto:David.Tesch@gov.bc.ca]

Sent: December 11, 2017 9:12 AM

To: Russell, Ken (EC)
Cc: Austin, Joyce ENV:EX
Subject: PRV Sampling
Importance: High

Hi Ken,

My name is David Tesch and I am Joyce's Executive Director. Joyce is not in the office today and my DM is asking if there is an ETA on the results from the PRV sampling that was done at the fish farms early last week. Are you able to provide me an answer?

Regards,
David Tesch
Executive Director
Knowledge Management Branch
Ministry of Environment & Climate Change Strategy
778-698-4406
David.Tesch@gov.bc.ca<mailto:David.Tesch@gov.bc.ca>

s.16(2)(c)

### Dickie, Catherine

From:

Lowe, Carmel

Sent:

December 14, 2017 6:29 PM

To:

Moore, Wayne; Taylor, Nathan

Subject:

Re: Request for call re: SSHI data

Hope we can add this to our discussion on Monday....

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Moore, Wayne

Sent: Thursday, December 14, 2017 17:22

To: Taylor, Nathan; Lowe, Carmel

Subject: Re: Request for call re: SSHI data

I suspect that there thinking is that it seems ever time something bad is found (eg, hsmi piece) the datat is released with an article but that all the other findings (ie, the data that led her to stop testing for reportables) never seems to get share/published. I suspect there concern is they would like to see more transperency.

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Taylor, Nathan

Sent: Thursday, December 14, 2017 8:09 PM

To: Lowe, Carmel; Moore, Wayne

Subject: RE: Request for call re: SSHI data

No clue I'm afraid and there are many possibilities.

From: Lowe, Carmel

Sent: Thursday, December 14, 2017 5:06 PM

To: Taylor, Nathan; Moore, Wayne

Subject: FW: Request for call re: SSHI data

Do either of you know what data he might be referring to and any plans for its release?

### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel.Lowe@dfo-mpo.qc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360

Government of Canada | Gouvernement du Canada

From: Reid, Rebecca

Sent: Thursday, December 14, 2017 4:02 PM

To: Lowe, Carmel <a href="mailto:carmel.towe@dfo-mpo.gc.ca">carmel.towe@dfo-mpo.gc.ca</a>

Cc: Johal, Sharan <a href="mailto-sharan\_Johal@dfo-mpo.gc.ca">sharan\_Johal@dfo-mpo.gc.ca</a>

Subject: FW: Request for call re: SSHI data

Can you advise on this? I'm not familiar with the details.
Thanks.
RR
Rebecca Reid Regional Director General/ Directrice générale régionale Fisheries and Oceans Canada - Pacific Region/ Pêches et Océans Canada - Région du Pacifique 200-401 Burrard Street / 401, rue Burrard, bureau 200 Vancouver, BC/CB V6C 3S4 Office / Téléphone: 604-666-6098 Cell / Cellulaire: E-mail/ Courriel: rebecca.reid@dfo-mpo.gc.ca
From: Sent: Thursday, December 14, 2017 3:43 PM To: Reid, Rebecca < Rebecca.Reid@dfo-mpo.gc.ca> Cc: Subject: Request for call re: SSHI data
Hi Rebecca,
I'm hoping you might be available for a call with and myself. We are hopeful that you might be able to help expedite the release of data held by the Strategic Salmon Health Initiative research team. We understand this is a collaborative research project, but DFO is doing the primary diagnostics. Given the paper published yesterday by Morton and Routledge we believe this data is important from a public perspective, as well as an aquaculture management perspective.
Please let us know if you can be available, I don't think we would need more than 30 minutes.
BC Salmon Farmers Association Office: (250) 286-1636 x Mobile:  BCSalmonFarmers.ca
Twitter:
Disclaimer: The contents of this email and any attachments are confidential to the intended recipient and must not be used or copied to unauthorized third parties in any way. If you are not the intended recipient, please notify the sender and delete the

ie intended recipient, please notify the sender and delete the

s.16(2)(c) s.19(1)

message and its content immediately.

### Dickie, Catherine

From:

Miller-Saunders, Kristi

Sent:

December 18, 2017 8:53 AM

To:

MacDougall, Lesley; Lowe, Carmel; Taylor, Nathan

Subject:

RE: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

I am good with the change

Kristi

----Original Message---From: MacDougall, Lesley
Sent: December-18-17 8:18 AM

To: Lowe, Carmel; Miller-Saunders, Kristi; Taylor, Nathan

Subject: RE: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

HI all; I agree with Carmel's change as well

Kristi - as soon as we've heard from you I can get a final copy to Carmel for approval

Cheers Lesley

----Original Message-----From: Lowe, Carmel

Sent: 2017-December-18 7:03 AM

To: Miller-Saunders, Kristi; MacDougall, Lesley; Taylor, Nathan Subject: Re: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

Thanks Lesley and Kristi. Nathan - I didn't hear from you so assuming you are comfortable...

Rather than Lesley's suggestion, I propose the following addition to final context piece:

'Further Science studies would be required to determine and evaluate any associated marine environmental or public health risks associated with such effluent discharges."

Comments before 9:30 am please.

Carmel

Sent from my BlackBerry 10 smartphone on the Rogers network.

**Original Message** 

From: Miller-Saunders, Kristi

Sent: Saturday, December 16, 2017 11:10

To: MacDougall, Lesley; Lowe, Carmel; Taylor, Nathan

Subject: RE: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

Here is a final copy with all tracking and comments removed.

Kristi

From: MacDougall, Lesley

Sent: December 16, 2017 9:55 AM

To: Lowe, Carmel; Taylor, Nathan; Miller-Saunders, Kristi

Subject: FW: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

Hi all, much improved, very minor changes suggested to identify where the genetics lab is.

Otherwise, I am good with this version: one Q - would it be useful in the final context piece to note that we don't really

have anything to compare the PRV loads to?

L

From: Lesley MacDougall

Sent: Saturday, December 16, 2017 9:47 AM

To: MacDougall, Lesley

Subject: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

s.19(1)

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-15-17 7:50 AM

To:

Parsons, Jay

Subject:

Re: The effect of exposure to farmed salmon on piscine orthoreovirus infection and

fitness in wild Pacific salmon in British Columbia, Canada

Thx

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Parsons, Jay

Sent: Friday, December 15, 2017 7:30 AM

To: Moore, Wayne

**Cc:** Lowe, Carmel; White, Andrea; Taylor, Nathan; Kennedy, Eddy; Johnson, Stewart; Bungay, Alfred; Burgetz, Ingrid **Subject:** RE: The effect of exposure to farmed salmon on piscine orthoreovirus infection and fitness in wild Pacific

salmon in British Columbia, Canada

Wayne,

Please find attached a short review of the PRV paper by Morton et al. which was published on December 13<sup>th</sup>, 2017. If you require a more thorough review of the paper and additional information on our ongoing research on PRV please let us know.

Thanks to Stewart, Kyle, Simon, and others for helping to put these comments together on short notice.

Jay

From: Moore, Wayne

**Sent:** December-14-17 8:32 AM **To:** Bungay, Alfred; Parsons, Jay **Cc:** Lowe, Carmel; White, Andrea

Subject: Fw: The effect of exposure to farmed salmon on piscine orthoreovirus infection and fitness in wild Pacific

salmon in British Columbia, Canada

Need a summary and observations plus link to our work today cob

### Parsons, Jay

From:

Moore, Wayne

Sent:

Friday, December 15, 2017 9:43 AM

To:

McPherson, Arran; Lowe, Carmel; LaRue, Jean-François

Cc:

Taylor, Nathan; Garver, Kyle; Johnson, Stewart; Jones, Simon; Parsons, Jay

Subject:

Review of Morton article

Please find below a review of the Morton article prepared by Jay's team with input from regional scientists (particularly, Kyle, Simon, and Stewart). Thanks to all for the timely work.

**....** ....

### Summary of: The effect of exposure to farmed salmon on piscine orthoreovirus infection and fitness in wild Pacific salmon in British Columbia, Canada

A new paper published in the open access journal PlosOne had the objective of conducting an analysis fish health data from BC farmed Atlantic Salmon and wild Pacific salmon to determine if there is a linked between PRV infections in wild and farmed salmon. This was assessed by analysing farmed fish obtained from fish market stores and opportunistically sampling wild salmon from regions defined as "high or low exposure" to salmon farms.

The authors are suggesting that their results indicate that PRV transfer is occurring from farmed Atlantic salmon to wild Pacific salmon, that infection in farmed salmon may be influencing infection rates in wild salmon, and that this may pose a risk of reduced fitness in wild salmon impacting their survival and reproduction. However, they do note in their conclusions that "The evidence, based solely on molecular screening tests from this observational study, and constrained by limited access to farmed Atlantic salmon samples of known provenance, cannot be definitive."

### General comments

It is important to highlight a number of points and assumptions arising from this study that may lead to a different interpretation of the findings and conclusions, including that the farmed salmon samples were obtained from fish market stores and thus the actual origin of the farmed fish is unknown.

It is already known in the literature that PRV infections occur in farmed Atlantic salmon and wild Pacific salmon in BC and that there can be differences in PRV infections between species differences and between stocks. However, in their analyses, it is not clear how the data on Pacific salmon have been combined or if it is even the same species composition or stock composition among their samples or areas. Hence, reported difference between areas could actually be attributable to factors other than "area".

The title of the paper and the article assert that the authors are examining the "fitness" in wild Pacific salmon. However, they are not actually examining fitness. Rather they are simply suggesting that because there may be a link between farmed salmon PRV infections and infections in wild salmon, and that based on observations of the impacts of PRV on farmed Norwegian salmon, they are suggesting there may be similar

impacts on wild Pacific salmon in BC and this <u>may</u> lead to impacts on "fitness". The current body of knowledge on PRV in BC nor the findings of their own study (including their own concluding comments that the evidence is not definitive), do not support any conclusive conclusions from this study.

The comments reported in the news article does not accurately reflect the contents of the paper. A more detailed analysis of the paper and comparison of comments in the news article versus the paper is below.

**CBC Article:** The research is the first of its kind to conclude that large numbers of B.C's wild salmon are becoming infected with PRV through exposure to fish farms.

Journal Article The author states: "PRV infection was highest among the farmed salmon categories; Atlantic salmon (95%) and steelhead (69%). The highest proportions of PRV-infected wild salmonids were from the high exposure regions, i.e., Regions 5–8, including the lake with a steelhead farm and the highly exposed inshore archipelago environments (37–50%). The proportion of PRV infection declined between the highly exposed lower (41%) and upper (22%) Fraser River. The lowest proportions were in Regions 1 and 2, furthest from salmon farms (5%). In addition, Cultus Lake trout were highly infected with PRV (76%) (Lake c, Fig 1), while only 3% of the salmonids in Oweekeno Lake were infected with PRV (Lake a, Fig 1, S1 Table)."

**CBC Article:** According to the research, PRV was found to be much more prevalent in the lower Fraser River than the upper Fraser River. "This suggests that salmon infected with PRV are less capable of swimming up through strong rapids in places like Hell's Gate and therefore unable to reach their spawning grounds," said coauthor Rick Routledge, a professor of statistics at Simon Fraser University."

**CBC** Article: The data also show that the virus makes it more difficult for wild salmon to swim upstream to their spawning grounds, which has major implications for the sustainability of the populations.

Journal Article: The authors state: "there was over a six-fold decline in the estimated proportion of PRV-positive test results from (a) fish in the low-challenge category to (b) those in the high-challenge category. This estimated decline is commensurate with the observed declines (i) between Regions 8 and 9 (the lower and upper Fraser River areas) and (ii) between the lower and higher elevations in Region 1 in northern BC (Table 3)" In the Fraser River, sites within the low-challenge category are below Hells Gate and the high-challenge category sites above Hells Gate. In the case of Skeena River site above major river constrictions are considered high-challenge sites.

### Our Concerns:

Upon reviewing their data (Supplementary Table 3) we noticed that there are large differences in the species of salmon tested for PRV between Areas. As an example: In the migration study which compared PRV loads between Area 8 (low challenge) and Area 9 (high challenge) in the Fraser River. Samples in Area 8 were a mix of trout and salmon species (44% Trout/Steelhead, 44% Pink and 8% Chinook salmon, 3% Chum and Sockeye, each), whereas samples from Area 9 were primarily Sockeye (96%) and Chinook Salmon (4%). Similar differences in species composition are seen between the other areas, as well as between years.

We feel that analysis of PRV infection using mixed hosts is a serious flaw in this paper as this assumes that all species have the same likelihood/susceptibility to infection with PRV which we know from published literature is not the case. A recent survey of salmon from Washington State and Alaska by Purcell et al. (2017) reported that findings of PRV RNA were most common in Coho and Chinook salmon. In addition to differences between species differences in age, time in seawater etc. will impact on the risk of PRV infection. It is known for farmed fish that the longer fish are in seawater the higher likelihood they become infected with PRV. Purcell et al. (2017) Fish Dis. 2017;1–9

# Other Concerns:

- 1. The authors fail to provide, with exception of Owekeno Lake, data on the amount/load of PRV present in their samples.
- 2. The authors do not provide any biological data on the hosts (e.g. date of capture, size, age) which would have help to inform the reader about residence time in seawater.
- 3. In the case of Area 3 it is likely that some of the fish that they sampled would have originated from systems entering into the Strait of Georgian and therefore would have had to have migrated past salmon farms. This paper doesn't consider aspects of host biology which we feel are critical to the interpretation of their results.
- 4. They include Area 4 (outer coast of Vancouver Island) as a low farm exposure sites, although 2 sites from which they obtained samples were from an area of salmon farming.

In their conclusions they state: "The evidence, based solely on molecular screening tests from this observational study, and constrained by limited access to farmed Atlantic salmon samples of known provenance, cannot be definitive. Nonetheless, we view it as providing an early warning sign of a potentially serious problem that warrants immediate and ongoing research. Research into the fitness impacts to wild Pacific salmonids of farmed salmon pathogens is needed in wild fish populations in addition to controlled laboratory environments, and could provide valuable insights useful for the management of critically declining wild salmon populations"

# **Ongoing DFO Research Efforts**

Research on PRV is ongoing at the Pacific Biological Station. Current work includes an ACRDP-funded project lead by Dr. Kyle Garver (PBS-Science) and Dr. A.P. Farrell (UBC). This project is examining the effects of PRV on physiological performance of Atlantic and Sockeye Salmon. There are also several projects under PARR lead by Dr. Garver investigating linkage between PRV and HSMI and impacts on Pacific salmon. And applications have been made by Drs. Garver and Johnson for funding to examine sources/reservoirs of PRV and survival/distribution of PRV in the environment.

#### Wayne Moore

Director General, Strategic and Regulatory Science Fisheries and Oceans Canada / Government of Canada Wayne.Moore@dfo-mpo.gc.ca / Tel: 613-990-0001

Directeur général, Sciences stratégiques et réglementaires

Pêches et Océans Canada / Gouvernement du Canada Wayne.Moore@dfo-mpo.gc.ca / Tél. : 613-990-0001

Web: <u>DFO/MPO</u>
Twitter: <u>DFO/MPO/DFO-Science/MPO-Science</u>

# Ryan, Patricia

From:

Moore, Wayne

Sent:

December-15-17 2:29 PM

To:

Taylor, Nathan

Subject:

Re: could you please call me?

Will call in 30

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Taylor, Nathan

Sent: Friday, December 15, 2017 2:10 PM

To: Moore, Wayne

Subject: could you please call me?

When you got a sec to talk about media lines etc.?

Nathan G. Taylor, Ph.D. Division Manager | Dire

Division Manager | Directeur de secteur

Aquatic Diagnostics Genomics and Technology Division | Division des diagnostics, la genomique, de la technologie aquatique

Fisheries and Oceans Canada | Peches et Oceans Canada Pacific Biological Station | Station biologique du Pacifique 250-756-7395

# Ryan, Patricia

From:

Moore, Wayne

Sent:

December-15-17 3:16 PM

To:

Taylor, Nathan

Subject:

Re: FOR URGENT APPROVAL: MLs of Effluent Testing

Categories:

ATIP

You are on line 6139900001

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Taylor, Nathan

Sent: Friday, December 15, 2017 3:09 PM

To: Moore, Wayne

Subject: RE: FOR URGENT APPROVAL: MLs of Effluent Testing

I'd appreciate the call anyway!

From: Moore, Wayne

Sent: Friday, December 15, 2017 12:09 PM

To: Taylor, Nathan

Subject: Re: FOR URGENT APPROVAL: MLs of Effluent Testing

Ok.

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Taylor, Nathan

Sent: Friday, December 15, 2017 2:46 PM

To: Moore, Wayne

Subject: RE: FOR URGENT APPROVAL: MLs of Effluent Testing

I certainly agree with that – there's some other information that Michelle sent up that's just plain wrong that needs to be fixed hence the reason for this emai/call

From: Moore, Wayne

Sent: Friday, December 15, 2017 11:45 AM

To: Taylor, Nathan

Subject: Fw: FOR URGENT APPROVAL: MLs of Effluent Testing

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Fagan, Ashley < Ashley.Fagan@dfo-mpo.qc.ca>

Sent: Friday, December 15, 2017 2:38 PM

To: McPherson, Arran; Morel, Philippe; Moore, Wayne; LaRue, Jean-François

Cc: White, Andrea; Richter, Julie; Villeneuve, Anne-Marie; Smith, Kathleen; Jenkins, Phil; Chow, Vance; Girouard, Louise;

Rainer, Michelle; Saindon, Carole

Subject: RE. FOR LIRGENT APPROVAL. MI's of Effluent Testing

Ok thanks Arran, we'll remove those two lines.

From: McPherson, Arran

**Sent:** December-15-17 2:34 PM

To: Fagan, Ashley; Morel, Philippe; Moore, Wayne; LaRue, Jean-François

Cc: White, Andrea; Richter, Julie; Villeneuve, Anne-Marie; Smith, Kathleen; Jenkins, Phil; Chow, Vance; Girouard, Louise;

Rainer, Michelle; Saindon, Carole

Subject: RE: FOR URGENT APPROVAL: MLs of Effluent Testing

Hi, I really question the need to include the following 2 bullets

Further, have we clarified if there is any issue with our release (through CSAS) of the report we provide to the province? I think this is the next question that would be asked. Arran.

From: Fagan, Ashley

Sent: Friday, December 15, 2017 2:09 PM

To: Morel, Philippe < <a href="mailto:Philippe.Morel@dfo-mpo.gc.ca">Philippe.Morel@dfo-mpo.gc.ca</a>; Moore, Wayne < <a href="mailto:Wayne.Moore@dfo-mpo.gc.ca">Wayne.Moore@dfo-mpo.gc.ca</a>; McPherson,

Arran < <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran <a href="mailto:Arran.Mc

Cc: White, Andrea <<u>Andrea.White@dfo-mpo.gc.ca</u>>; Richter, Julie <<u>Julie.Richter@dfo-mpo.gc.ca</u>>; Villeneuve, Anne-Marie <<u>Anne-Marie.Villeneuve@dfo-mpo.gc.ca</u>>; Smith, Kathleen <<u>Kathleen.Smith@dfo-mpo.gc.ca</u>>; Jenkins, Phil <<u>Phil.Jenkins@dfo-mpo.gc.ca</u>>; Chow, Vance <<u>Vance.Chow@dfo-mpo.gc.ca</u>>; Girouard, Louise <<u>Louise.Girouard@dfo-mpo.gc.ca</u>>; Rainer, Michelle <<u>Michelle.Rainer@dfo-mpo.gc.ca</u>>; Saindon, Carole <<u>Carole.Saindon@dfo-mpo.gc.ca</u>>

Subject: RE: FOR URGENT APPROVAL: MLs of Effluent Testing

Thank you. We are moving these to DMO.

From: Morel, Philippe

Sent: December-15-17 2:04 PM

To: Moore, Wayne; Fagan, Ashley; McPherson, Arran; LaRue, Jean-François

Cc: White, Andrea; Richter, Julie; Villeneuve, Anne-Marie; Smith, Kathleen; Jenkins, Phil; Chow, Vance; Girouard, Louise;

Rainer, Michelle; Saindon, Carole

Subject: RE: FOR URGENT APPROVAL: MLs of Effluent Testing

Agree and approved

s.21(1)(a)

s.21(1)(b)

Philippe

De: Moore, Wayne

Envoyé: 15 décembre 2017 13:56

À: Fagan, Ashley < Ashley. Fagan@dfo-mpo.gc.ca >; McPherson, Arran < Arran. McPherson@dfo-mpo.gc.ca >; Morel,

Philippe < Philippe. Morel@dfo-mpo.gc.ca>; LaRue, Jean-François < Jean-François.LaRue@dfo-mpo.gc.ca>

**Cc:** White, Andrea < <u>Andrea.White@dfo-mpo.gc.ca</u>>; Richter, Julie < <u>Julie.Richter@dfo-mpo.gc.ca</u>>; Villeneuve, Anne-Marie < <u>Anne-Marie.Villeneuve@dfo-mpo.gc.ca</u>>; Smith, Kathleen < <u>Kathleen.Smith@dfo-mpo.gc.ca</u>>; Jenkins, Phil

<Phil.Jenkins@dfo-mpo.gc.ca>; Chow, Vance <<u>Vance.Chow@dfo-mpo.gc.ca</u>>; Girouard, Louise <<u>Louise.Girouard@dfo-mpo.gc.ca</u>>; Rainer, Michelle <<u>Michelle.Rainer@dfo-mpo.gc.ca</u>>; Saindon, Carole <<u>Carole.Saindon@dfo-mpo.gc.ca</u>>
Objet: Re: FOR URGENT APPROVAL: MLs of Effluent Testing

Mitigate "any" risk to the environment. Otherwise ok

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Fagan, Ashley

Sent: Friday, December 15, 2017 1:49 PM

To: McPherson, Arran; Moore, Wayne; Morel, Philippe; LaRue, Jean-François

Cc: White, Andrea; Richter, Julie; Villeneuve, Anne-Marie; Smith, Kathleen; Jenkins, Phil; Chow, Vance; Girouard, Louise;

Rainer, Michelle; Saindon, Carole

Subject: FOR URGENT APPROVAL: MLs of Effluent Testing

Arran, Wayne, Philippe, JF:

MINO has urgently requested the below media lines for **2pm**. Please let us know **ASAP** if you have any concerns as we'll need to move this to DMO shortly. Approved by Carmel Lowe and Rebecca Reid.

Thanks,

Ashley

Issue: In November, a video that shows blood and other effluent coming from a farmed fish processing plant in Brown's Bay, BC was released. The video has received considerable media attention and has raised concerns about the possible presence of disease and pathogens in the discharge, particularly PRV. Media lines are required to explain DFO's role in testing and analyzing samples from the effluent collected from two BC fish processing plants.

Deadline: ASAP

Recommendation: A proactive approach is recommended with Carmel Lowe, RD Science, as spokesperson

Approved by: Carmel Lowe, Rebecca Reid

#### Media lines:

- At the request of the BC Ministry of Environment & Climate Change Strategies (ENV), Fisheries and Oceans Canada (DFO) is analyzing samples that collected from two BC fish processing plants.
- The samples are being analyzed for the presence or absence of piscine reovirus (PRV) at the DFO Forensics Laboratory at the Pacific Biological Station in Nanaimo, BC.
- This is a secure, restricted-access and restricted-use laboratory, where strict protocols are followed to ensure sample integrity.
- The Province of BC, the Canadian Food Inspection Agency, and Environment and Climate Change Canada and are responsible for the licensing and regulation of fish processing facilities and related effluent. DFO has the lead federal role in managing Canada's fisheries and supports sustainable aquatic ecosystems through habitat protection, oceans management and ecosystems research.

s.21(1)(a)

s.21(1)(b)

Ashley Fagan

Senior Communications Advisor | Conseillère principale en communications

Fisheries and Oceans Canada | Pêches et Océans Canada ashley.fagan@dfo-mpo.gc.ca 613-990-9415

# Dickie, Catherine

From:

MacDougall, Lesley

Sent:

December 16, 2017 9:55 AM

To:

Lowe, Carmel; Taylor, Nathan; Miller-Saunders, Kristi

Subject:

FW: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

Attachments:

RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final.docx

Hi all, much improved, very minor changes suggested to identify where the genetics lab is.

Otherwise, I am good with this version: one Q - would it be useful in the final context piece to note that we don't really have anything to compare the PRV loads to?

L

From: Lesley MacDougall

Sent: Saturday, December 16, 2017 9:47 AM

To: MacDougall, Lesley

Subject: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

# Centre for Science Advice Pacific

FPP non-CSAS Request for Rapid Science Response

	Request Contact:	Qriefagriggrenie	Project Type: Aquaculture-Emergency response
********	Date of request: footprint:	Cas. 2006 28- Julian 1986 - a 251, <b>2017</b>	Project
	Region of proposed impact:	Tofino and Browns Bay, British Columbia	Habitat Type: Coastal
	Purpose of request:	Information for Ministry of Environment / En	vironment and Climate Change Canada investigation
	Potential affected species:	Pacific salmon	
	Date required:	December 15, 2017	Request #:2017AQU01
			and the second s

Timeline rationale

#### PROJECT OVERVIEW

In Canada, the regulation of processing of fish products is a shared Provincial — Federal responsibility. BC Ministry of Environment & Climate Change Strategies (ENV) supports marine fisheries and aquaculture and seafood industry development, issuing and licenses to businesses involved in the aquaculture sector - including permits for seafood processing facilities - under the BC Fisheries Act and Fish Inspection Act. While the conditions of the clicense include the requirement to construct and operate the facility to ensure that activities are conducted in a manner that will prevent fish and aquatic plants from becoming unsafe food, there is no prohibition of the discharge of fish waste effluent, or requirement of specific treatment of fish waste effluent prior to discharge into the environment. Thus, as a function of normally-operating fish processing plants, fish waste effluent is released back into the marine ecosystem subject to conditions of the processing plants, fish waste effluent is released back into the marine ecosystem subject to conditions of

Environment and Climate Change Canada (ECCC) administers section 36 of DFO's Fisheries Act, the key pollution prevention provision, prohibiting the deposit of deleterious substances into waters frequented by fish, unless authorized by regulations under the Fisheries Act or other federal legislation. A deleterious substance can be any substance that, if added to any water, would degrade or alter its quality such that it could be harmful to fish, fish habitat or the use of fish by people.

Following the public release of a video showing fish waste effluent being released into the marine environment, and subsequent news articles and public interest, ENV initiated an inspection of two fish processing plants that are currently the focus of investigation. ENV also has an upcoming compliance audit for fish processing plants.

The BC Ministry of Environment & Climate Change Strategies (ENV), Environmental Protection Regional Operations Branch requested assistance from DFO to support the collection of water samples at two fish processing facilities, and to test collected effluent for the presence of the Piscine Reovirus (PRV), as part of the ongoing inspection. Other labs contacted at the time did not have the capacity to perform the necessary diagnostics on water samples.

Comment [1]: Send to AMD to ensure accuracy

#### Background:

Piscine OrthoReovirus (PRV) is known to be present in Norway, Japan, the United Kingdom, Ireland, Chile, the United States and Canada (Biering and Garseth 2012; Kibenge et al. 2013; Siah et al. 2015). Farmed and wild Atlantic Salmon (Salmo salar), Coho Salmon (Oncorhynchus kisutch), Chinook Salmon (Oncorhynchus tshawytscha), and Rainbow trout (Oncorhynchus mykiss), and wild Cutthroat Trout (Oncorhynchus clarkii), Steelhead Trout (Oncorhynchus mykiss), Sockeye Salmon (Oncorhynchus nerka), Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) have all tested positive for PRV through molecular testing. However, around the world, PRV prevalence has been substantially higher in farmed populations, most showing 60-90+% prevalence, than in wild populations.

the context with this we are accessed in the constant of the confidence of the best of the best of the constant of the constan

PRV is causative of Heart and Skeletal Muscle Inflammation (HSMI) disease in farmed Atlantic Salmon (Wessell et al. 2017) and EIBS disease in farmed Coho salmon in Japan (Takano et al 2016). As well, it has been implicated in other diseases of farmed Rainbow Trout, Coho and Chinook Salmon in Chile (Godoy et al. 2016), Norway (Hauge et al. 2017) and Canada (Miller et al. 2017). HSMI disease occurs in farmed Atlantic salmon in Norway (Kongtorp et al. 2004), Scotland (Ferguson et al. 2005), Chile (Godoy et al. 2016), and Canada (Di Cicco et al. 2017).

Red blood cells (RBCs) are the primary infective tissue for PRV (RED DESCRIPTION OF ALL 2014). Not all salmon carrying the virus, even at high load, are diseased. Given the high prevalence of PRV in farmed populations and presence of the virus in the blood, it was expected that blood effluent from processing plants would contain PRV.

There is a large body of literature that has established the infectivity of PRV in Norway and its co-occurrence with the disease HSMI. More recently, DFO scientists have been investigating the infectivity and disease causing potential of the strain of PRV present in BC. Their studies have demonstrated that PRV from BC can infect Sockeye, Chinook, and Atlantic Salmon.

After infection, PRV can reach high levels in the blood and is capable of being present for many months; these findings are similar to those from Norwegian challenge studies, however, recent studies indicate that there is uncertainty regarding the potential ability of PRV to cause disease in BC.

DFQ scientists, along with provincial and international colleagues, are conducting investigations to better understand the biology of PRV and Heart and Skeletal Muscle inflammation (HSMI) disease in wild and farmed salmon on the west coast of North America. To date the disease HSMI always occurs in the presence of PRV. While there have been other agents in addition to PRV which have been found in fish with HSMI disease, researchers agree that PRV is one of the leading candidates to be a causative agent.

A study documenting the first farm level diagnosis of HSMI in BC was recently published (Di Cicco et al. 2017). The study showed inflammatory lesions in heart and skeletal muscle tissue diagnostic of this disease in a longitudinal study from one Atlantic Salmon farm in BC. At an individual level, not all fish carried both heart and skeletal lesions at any given point in time, but at the farm level, both were present and diagnostic of the disease.

Request:

Comment [2]: DFO 2015 is no longer sufficiently accurate, and there are more farmed and wild salmon have been testing than intimated.

Comment [3]: You simply have to make some statement about linkages with disease.

Comment [4]:
This deviates from the website information – and must be substantiated

As	part	of	an	inspection	conducte	i at	two	fish	processing	facilities,	ENV	requested	DFO	expertise t	(0)

- Accompany 1 ENV and 1 ECCC inspector to two fish processing facilities and support the collection of water camples:
- Analyze effluent samples collected from two fish processing plants on December 4 and 5, 2017; and

Importance:	∠ Essential	☐ Important	☐ Desirable	
		Science	Response	

#### Methods:

A DFO Molecular Genetics technician accompanied two inspectors (from ENV and ECCC) to each of the fish processing facilities to provide guidance for the appropriate collection of water samples to allow for viral analysis. Also present were for the Senior Enforcement Officer - Enforcement Branch Environment and Climate Change Canada, and the Environmental Protection Officer - Compliance Section, Environmental Protection Division, BC Ministry of Environment and Climate Change Strategy.

At the Browns Bay Packing Co, Campbell River, BC, effluent was collected from one source — at the plant itself, after it had been passed over a roto-screen of 0.5 mm and 0.25 mm (as per their permit), and immediately prior to discharge out of the facility into the environment. This sample was the combination of discharge from the fish transport vessel (a mixture of saltwater, ice-water and blood from the fish being processed) and the liquid discharge from the plant used in the processing of the fish. The sample was red in colour, with very small particulates visible. This is MGL sample 2017-0017-J3205.

At the Lions Gate Fish Co. Tofino, BC, effluent was collected from two sources;

- 1) acceptable Discharge from the fish harvest vessel (a mixture of saltwater, ice-water and blood from the fish being processed). The vessel discharge is un-treated and enters the fish plant discharge pipe after the plant discharge is screened, and so was considered a separate sample by the accompanying inspectors. The sample was bright red in colour, and fairly clear with very little particulate matter visible. This is MGL sample 2017-0017-J3231
- 2) discharge from the processing plant that was used in the processing and cleaning of fish. This sample was collected after it had passed over a fine-meshed screen (as per their permit) and prior to discharge out into the environment. This sample was had a cloudy, white appearance, with visible particulate matter. This is MGL sample 2017-0017-J3232

Note – one additional discharge from this site was unable to be accessed for sampling. The liquid from the processing plant floor is passed over a 6mm screen, and then joins directly into the pipe for discharge.

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#### Laboratory

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#### Sample Handling

Samples were collected in new 2L sample bottles, and were transported to the <u>DFO</u> Molecular Genetics Lab on ice and in a locked truck. They arrived at the lab within 3-6 hours of collection.

Samples from the Fish Plant discharge inspections (MGL AQ# 2017-0017) were to receive processed in the Molecular Genetics Forensics Lab at the Pacific Biological station,

that is dedicated to highly sensitive and/or legal samples. This room is kept locked at all times, and has extremely limited access to qualified MGL staff. All personnel accessing this lab are required to sign in/out. Samples processed in this lab are either of a legal nature, or are highly sensitive or degraded DNA (e.g. eDNA/Scat samples). Only one sample file/type can be processed at one time in this lab, and the laboratory processing stations are sterilized with bleach and UV between samples. Any processing steps requiring use of equipment outside of this laboratory were carried out with samples constrained to sealed containers (e.g. centrifugation, PCR amplification).

Details of sample processing methodologies are presented in Appendix 1. Some equipment and element of the expension of the ex

Sampla Preservation

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#### Results:

PRV was detected in all samples tested. The number of viruses per mL of water was calculated to allow total viral burden to be assessed based on discharge estimates. Technical controls confirmed that equipment was performing appropriately and results were of high integrity.

Detailed results are presented in Appendix 2A (raw data containing results for all technical replicates) and 28 (synthesized data).

#### Context:

The high prevalence of PRV in farmed BC Salmon is well documented. The presence of the virus in facility effluent was therefore expected.

#### References:

Biering E., Garseth A.H. 2012. Heart and Skeletal Muscle Inflammation (HSMI) of farmed Atlantic Salmon (Salmo salar L.) and the associated Piscine Reovirus (PRV). In: Feist S, editor. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. Copenhagen: International Council for the Exploration of the Sea; p. 6.

Di Cicco, E., Ferguson, H.W., Schulze, A.D., Kaukinen, K.H., Li, S., Vanderstichel, R., Wessel, O., Rimstad, E., Gardner, I.A., Hammell, K.L., 2017, Heart and skeletal muscle inflammation (HSMI) disease diagnosed on a British Columbia salmon farm through a longitudinal farm study. PLoS ONE 12, 843 e0171471.

Ferguson, H.W., Kongtorp, R.T., Taksdal, T., Graham, D. and Falk, K., 2005. An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, Salmo salar L., with observations on myocardial regeneration. Journal of fish diseases, 28(2), pp.119-123.

Finstad, O.W., Dahle, M.K., Lindholm, T.H., Nyman, I.B., Lovoll, M., Wallace, C., Olsen, C.M., Storset, A.K. and Rimstad, E., 2014. Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. *Veterinary research*, 45(1), p.35.

Godoy M.G., Kibenge M.J., Wang Y., Suarez R., Leiva C., Vallejos F., Kibenge F.S. 2016. First description of clinical presentation of piscine orthoreovirus (PRV) infections in salmonid aquaculture in Chile and identification of a second genotype (Genotype II) of PRV. Virology journal 13(1): p.98.

Hauge H., Vendramin N., Taksdal T., Olsen A.B., Wessel Ø., Mikkelsen S.S., Alencar A.L.F., Olesen N.J., Dahle M.K. 2017. Infection experiments with novel Piscine orthoreovirus from rainbow trout (Oncorhynchus mykiss) in salmonids. PloS one 12(7), p.e0180293.

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Miller, K.M., Günther, O.P., Li, S., Kaukinen, K.H. and Ming, T.J., 2017. Molecular indices of viral disease development in wild migrating salmon. Conservation Physiology 5(1).

Siah A., Morrison D.B., Fringueili E., Savage P., Richmond Z., Johns R., Purcell M.K., Johnson S., C, Saksida S.M. 2015. Piscine reovirus: Genomic and molecular phylogenetic analysis from farmed and wild salmonids collected on the Canada/US Pacific Coast. PLoS ONE 10(11):e0141475.

Takano T., Nawata A., Sakai T., Matsuyama T., Ito T., Kurita J., Terashima S., Yasuike M., Nakamura Y., Fujiwara A., Kumagai A. 2016. Full-Genome sequencing and confirmation of the causative agent of Erythrocytic inclusion body syndrome in Coho Salmon identifies a new type of Piscine Orthoreovirus. PloS one 11(10), p.e0165424.

Wessel Ø., Braaen S., Alarcon M., Haatveit H., Roos N., Markussen T., Tengs T., Dahle M.K., Rimstad E. 2017. Infection with purified Piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. PloS one 12(8), p.e0183781.

Respon	der: XXXX, Science	Responder:
	does not constitute delivery rScience input.	of peer - reviewed Science advice; it is intended as a rapid response to an immediate
Reviewed by:	Lesley MacDougall, Coordin	ator, Centre for Science Advice Pacific Region
Date:	XXXX, 2017	
Comments:		

Approved by:	
Date:	
Comments:	

# Dickie, Catherine

From:

Miller-Saunders, Kristi

Sent:

December 16, 2017 11:11 AM

To:

MacDougall, Lesley; Lowe, Carmel; Taylor, Nathan

Subject:

RE: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

**Attachments:** 

RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final-AC.docx

Here is a final copy with all tracking and comments removed.

Kristi

From: MacDougall, Lesley

Sent: December 16, 2017 9:55 AM

To: Lowe, Carmel; Taylor, Nathan; Miller-Saunders, Kristi

Subject: FW: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

Hi all, much improved, very minor changes suggested to identify where the genetics lab is.

Otherwise, I am good with this version: one Q - would it be useful in the final context piece to note that we don't really

have anything to compare the PRV loads to?

1

From: Lesley MacDougall

Sent: Saturday, December 16, 2017 9:47 AM

To: MacDougall, Lesley

Subject: RSR2017\_AQU01\_Browns%20and%20TofinoPRV\_Final

# Centre for Science Advice Pacific

FPP non-CSAS Request for Rapid Science Response

Request Contact:	Donada peren	Project Type: Aquaculture-Emergency response
Date of request:	November 29, 2017	Project footprint:
Region of proposed impact:	Tofino and Browns Bay, British Columbia	Habitat Type: Coastal
Purpose of request:	Information for Ministry of Environment / En	vironment and Climate Change Canada investigation
Potential affected species:	Pacific salmon	and the first section of the section
Date required:	December 15, 2017	Request #:2017AQU01
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Timeline rationale:	gamen wenter	

#### PROJECT OVERVIEW

In Canada, the regulation of processing of fish products is a shared Provincial – Federal responsibility. BC Ministry of Environment & Climate Change Strategies (ENV) supports marine fisheries and aquaculture and seafood industry development, issuing licenses to businesses involved in the aquaculture sector - including permits for seafood processing facilities - under the BC Fisheries Act and Fish Inspection Act. Thus, as a function of normally-operating fish processing plants, fish waste effluent is released back into the marine ecosystem subject to conditions of license.

Environment and Climate Change Canada (ECCC) administers section 36 of DFO's *Fisheries Act*, the key pollution prevention provision, prohibiting the deposit of deleterious substances into waters frequented by fish, unless authorized by regulations under the *Fisheries Act* or other federal legislation. A deleterious substance can be any substance that, if added to any water, would degrade or alter its quality such that it could be harmful to fish, fish habitat or the use of fish by people.

Following the public release of a video showing fish waste effluent being released into the marine environment, and subsequent news articles and public interest, ENV initiated an inspection of two fish processing plants that are currently the focus of investigation. ENV also has an upcoming compliance audit for fish processing plants.

The BC Ministry of Environment & Climate Change Strategies (ENV), Environmental Protection Regional Operations Branch requested assistance from DFO to support the collection of water samples at two fish processing facilities, and to test collected effluent for the presence of the Piscine OrthoReovirus (PRV), as part of the ongoing inspection. Other labs contacted at the time did not have the capacity to perform the necessary diagnostics on water samples.

#### st 4. Ottestion

#### Background:

Piscine OrthoReovirus (PRV) is known to be present in Norway, Japan, the United Kingdom, Ireland, Chile, the United States and Canada (Biering and Garseth 2012; Kibenge et al. 2013; Siah et al. 2015). Farmed and wild Atlantic Salmon (Salmo salar), Coho Salmon (Oncorhynchus kisutch), Chinook Salmon (Oncorhynchus tshawytscha), and Rainbow trout

(Oncorhynchus mykiss), and wild Cutthroat Trout (Oncorhynchus clarkii), Steelhead Trout (Oncorhynchus mykiss), Sockeye Salmon (Oncorhynchus nerka), Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) have all tested positive for PRV through molecular testing. However, around the world, PRV prevalence has been substantially higher in farmed populations, most showing 60-90+% prevalence, than in wild populations.

PRV is causative of Heart and Skeletal Muscle Inflammation (HSMI) disease in farmed Atlantic Salmon (Wessell et al. 2017) and EIBS disease in farmed Coho salmon in Japan (Takano et al 2016). As well, it has been implicated in other diseases of farmed Rainbow Trout, Coho and Chinook Salmon in Chile (Godoy et al. 2016), Norway (Hauge et al. 2017) and Canada (Miller et al. 2017). HSMI disease occurs in farmed Atlantic salmon in Norway (Kongtorp et al. 2004), Scotland (Ferguson et al. 2005), Chile (Godoy et al. 2016), and Canada (Di Cicco et al. 2017).

Red blood cells (RBCs) are the primary infective tissue for PRV (Finstad et al. 2014). Not all salmon carrying the virus, even at high load, are diseased. Given the high prevalence of PRV in farmed populations and presence of the virus in the blood, it was expected that blood effluent from processing plants would contain PRV.

R			

As part of an inspection conducted at two fish processing facilities, ENV requested DFO expertise to:

	samples; Analyze effl	ed from two fish pr	processing facilities and support the collection of water ocessing plants on December 4 and 5, 2017; and	
mpc	rtance:	☐ Important	☐ Desirable	

#### SCIENCE RESPONSE

#### Methods:

A DFO Molecular Genetics technician accompanied two inspectors (from ENV and ECCC) to each of the fish processing facilities to provide guidance for the appropriate collection of water samples to allow for viral analysis. Also present were the Senior Enforcement Officer - Enforcement Branch Environment and Climate Change Canada, and the Environmental Protection Officer - Compliance Section, Environmental Protection Division, BC Ministry of Environment and Climate Change Strategy.

At the Browns Bay Packing Co, Campbell River, BC, effluent was collected from one source – at the plant itself, after it had been passed over a roto-screen of 0.5 mm and 0.25 mm (as per their permit), and immediately prior to discharge out of the facility into the environment. This sample was the combination of discharge from the fish transport vessel (a mixture of saltwater, ice-water and blood from the fish being processed) and the liquid discharge from the plant used in the processing of the fish. The sample was red in colour, with very small particulates visible. This is MGL sample 2017-0017-J3205.

At the Lions Gate Fish Co, Tofino, BC, effluent was collected from two sources;

- 1) Discharge from the fish harvest vessel (a mixture of saltwater, ice-water and blood from the fish being processed). The vessel discharge is un-treated and enters the fish plant discharge pipe after the plant discharge is screened, and so was considered a separate sample by the accompanying inspectors. The sample was bright red in colour, and fairly clear with very little particulate matter visible. This is MGL sample 2017-0017-J3231
- 2) Discharge from the processing plant that was used in the processing and cleaning of fish. This sample was collected after it had passed over a fine-meshed screen (as per their permit) and prior to discharge out into the environment. This sample was had a cloudy, white appearance, with visible particulate matter. This is MGL sample 2017-0017-J3232

Note – one additional discharge from this site was unable to be accessed for sampling. The liquid from the processing plant floor is passed over a 6mm screen, and then joins directly into the pipe for discharge.

#### **Laboratory Sample Handling**

Samples were collected in new 2L sample bottles, and were transported to the DFO Molecular Genetics Lab on ice and in a locked truck. They arrived at the lab within 3-6 hours of collection.

Samples from the Fish Plant discharge inspections (MGL AQ# 2017-0017) were processed in the Molecular Genetics Forensics Lab at the Pacific Biological station, a laboratory space in the Molecular Genetics Section of DFO Science that is dedicated to highly sensitive and/or legal samples. This room is kept locked at all times, and has extremely limited access to qualified MGL staff. All personnel accessing this lab are required to sign in/out. Samples processed in this lab are either of a legal nature, or are highly sensitive or degraded DNA (e.g. eDNA/Scat samples). Only one sample file/type can be processed at one time in this lab, and the laboratory processing stations are sterilized with bleach and UV between samples. Any processing steps requiring use of equipment outside of this laboratory were carried out with samples constrained to sealed containers (e.g. centrifugation, PCR amplification).

Details of sample processing methodologies are presented in Appendix 1.

#### Results:

PRV was detected in all samples tested. The number of viruses per mL of water was calculated to allow total viral burden to be assessed based on discharge estimates. Technical controls confirmed that equipment was performing appropriately and results were of high integrity.

Detailed results are presented in Appendix 2A (raw data containing results for all technical replicates) and 2B (synthesized data).

#### Context:

The high prevalence of PRV in farmed BC Salmon is well documented. The presence of the virus in facility effluent was therefore expected.

#### References:

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Miller, K.M., Günther, O.P., Li, S., Kaukinen, K.H. and Ming, T.J., 2017. Molecular indices of viral disease development in wild migrating salmon. Conservation Physiology 5(1).

Siah A., Morrison D.B., Fringuelli E., Savage P., Richmond Z., Johns R., Purcell M.K., Johnson S..C, Saksida S.M. 2015. Piscine reovirus: Genomic and molecular phylogenetic analysis from farmed and wild salmonids collected on the Canada/US Pacific Coast. PLoS ONE 10(11):e0141475.

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Wessel Ø., Braaen S., Alarcon M., Haatveit H., Roos N., Markussen T., Tengs T., Dahle M.K., Rimstad E. 2017. Infection with purified Piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. PloS one 12(8), p.e0183781.

Responder:	XXXX, Science	Responder:	

#### REVIEW INFORMATION

This response does not constitute delivery of peer – reviewed Science advice; it is intended as a rapid response to an immediate requirement for Science input.

Reviewed by:	esley MacDougall, Coordinator, Centre for Science Advice Pacific Region
Date:	XXXX, 2017
Comments:	
Approved by:	
Date:	
Comments:	

# **Burgetz, Ingrid**

Subject:

Risk Assessment - Path Forward (UPDATED)

Location:

12E238 & Teleconference

Start:

Mon 18/12/2017 11:00 AM

End:

Mon 18/12/2017 12:00 PM

**Show Time As:** 

Tentative

Recurrence:

(none)

**Meeting Status:** 

Not yet responded

Organizer:

Moore, Wayne

**Required Attendees:** 

Lowe, Carmel; LaRue, Jean-François; Parsons, Jay; Thomson, Andrew

Taylor, Nathan; Caroline Mimeault; Burgetz, Ingrid **Optional Attendees:** 



MECTS-#378767....







Systemic bacterial Leads for risk

infection w... assessments.xls... pathogen transf...

# **Teleconference Dial-In Information**

Local dial-in/Numéro de telephone local: (613) 960-7513

**Conference ID/Numéro de conférence:** 

Toll-free dial-in/ Numéro de telephone sans frais: 1-877-413-4788

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2017-007-00933 EKME # 3787671

MEMORANDUM FOR THE DIRECTOR GENERALS OF STRATEGIC AND REGULATORY SCIENCE, AQUACULTURE MANAGEMENT DIRECTORATE, PACIFIC REGION SCIENCE, AND PACIFIC FISHERIES MANAGEMENT

# UPDATE ON PATHOGEN TRANSFER RISK ASSESSMENTS IN THE DISCOVERY ISLANDS IN BRITISH COLUMBIA

(FOR INFORMATION)

#### **SUMMARY**

The purpose of this memo is to update you on the planned next steps for pathogen transfer risk assessments in the Discovery Islands in British Columbia.

Following the successful Canadian Science Advisory Secretariat peer review of the assessment of the risk to Fraser River sockeye salmon due to Infectious Hematopoietic Necrosis Virus transfer from Atlantic salmon farms in the Discovery Islands, the same framework will be applied to assessing the risks associated with other pathogens that have caused disease on Atlantic salmon farms in that area.

Nine pathogens have been identified to potentially undergo formal risk assessments, and in preparation for initiating the next risk assessments, the scientific information on the characteristics of each pathogen is being collated and analyzed. As much of the scientific expertise related to bacterial pathogens resides outside of the department, the timing of the next risk assessments will be dependent on the availability of these external experts. However, in order to meet the government's commitment to respond to the Cohen Commission recommendations, this series of risk assessments, including a synthesis, need to be completed by the end of the 2019/2020 fiscal year.

# **BACKGROUND**

In 2014, Aquaculture Management (NCR and Pacific) requested Canadian Science Advisory Secretariat (CSAS) science advice on the risks of pathogen transfer from Atlantic salmon farms to Pacific salmon in British Columbia. It was then agreed that, as a starting point, the risks to Fraser River sockeye salmon would be undertaken as a priority, consistent with the Cohen commission recommendations. In addition, it was also agreed that advice would be delivered through a series of pathogen-specific risk assessments, followed by a synthesis.

The first pathogen transfer risk assessment conducted under the Aquaculture Science Environmental Risk Assessment Initiative was successfully peer-reviewed through a Canadian Science Advisory Secretariat meeting in December 2016. This first assessment determined the risks to Fraser River sockeye salmon abundance and diversity due to infectious hematopoietic necrosis virus (IHNV) transfer from Atlantic salmon farms located in the Discovery Islands of British Columbia to be minimal. The peer-review process confirmed the risk assessment framework and conceptual model to be appropriate for conducting pathogen transfer risk assessments.

As agreed at the onset of this process, the Aquaculture Regulatory Science group is now planning the next pathogen transfer risk assessments in the Discovery Islands.

# STRATEGIC CONSIDERATIONS

The following three criteria have been established to prioritize the next pathogen transfer risk assessments to be conducted in the Discovery Islands area:

- 1. pathogen(s) cause disease on Atlantic salmon farms in the Discovery Islands;
- 2. sockeye salmon are susceptible to the disease caused by the pathogen; and
- 3. there is temporal overlap between disease outbreaks on Atlantic salmon farms and sockeye salmon occurrence in the Discovery Islands.

Evidence that some pathogens caused disease on Atlantic salmon farms in the Discovery Islands (first criteria) was obtained by evaluating data submitted by industry as fish health events and diagnosed at the farm level through fish health audits between 2002 and early 2017. In addition to IHNV for which a pathogen transfer risk assessment has already been completed, the following nine diseases have been reported on Atlantic salmon farms in the Discovery Islands:

- amoebic gill disease (AGD) caused by Neoparamoeba perurans (protozoa);
- bacterial kidney disease (BKD) caused by Renibacterium salmoninarum (bacteria);
- enteric redmouth disease (ERD) caused by Yersinia ruckeri (bacteria);
- furunculosis caused by Aeromonas salmonicida (bacteria);
- idiopathic heart disease of which the cause is unknown;
- mouth rot caused by Tenacibaculum maritimum (bacteria);
- salmonid rickettsial septicaemia (SRS) caused by Piscirickettsia salmonis (bacteria);
- viral hemorrhagic septicaemia (VHS) caused by the VHS virus; and
- winter ulcers caused by Moritella viscosa and other bacteria.

Six out the nine diseases are caused by bacterial pathogens for which there is a lack of expertise within the department. As a result, it will be necessary to engage with external experts to assist with the risk assessments of bacterial pathogens, and the timing of those risk assessments will consequently be dependent on the availability of those experts.

The assessment of the risks related to idiopathic heart disease will incorporate conclusions from recent publications and on-going research on heart and skeletal muscle inflammation (HSMI) and *Piscine orthoreovirus* (PRV).

Other diseases have been detected on Pacific salmon farms within the Discovery Islands, or on Atlantic salmon farms outside of the Discovery Islands, or have been identified to Aquaculture Management Directorate as being of concern to Environmental Non-Government Organizations. These diseases are (1) atypical furunculosis, (2) infectious salmon anemia (ISA), (3) *Microsporidium cerebralis* infection, (4) salmon leukemia and (5) vibriosis. Currently, the pathogens causing those diseases fall outside of the scope of this initiative which is limited to pathogens causing disease on Atlantic salmon farms in the Discovery Islands.

# INTERNAL CONSULTATIONS

The above lists were developed in collaboration with Aquaculture Management Directorate during a meeting with Aquaculture Regulatory Science and Aquaculture Management (NCR and Pacific) in Vancouver in May 2017 to discuss the Fish Health Research Plan, which included among other items, status and next steps on pathogen transfer risk assessments in the Discovery Islands.

Aquaculture Management agreed that Aquaculture Regulatory Science would determine the details of how the next risk assessments for the nine remaining pathogens that have caused disease on Atlantic salmon farms in the Discovery Islands will be conducted.

## **EXTERNAL CONSULTATIONS**

The results of the evaluation of pathogens that caused disease on Atlantic salmon farms in the Discovery Islands were confirmed with industry veterinarians. Additionally, an overview of the IHNV risk assessment has been presented to the members of the British Columbia Salmon Farmer Association (BCSFA).

## **NEXT STEPS**

The science advisory report and accompanying research documents from the IHNV risk assessment will be published on the Canadian Science Advisory Secretariat website this fall. A technical briefing with stakeholders is being planned just prior to publication to provide an overview of the risk assessment process and findings, and to discuss the next pathogens that will be addressed.

.../4

Aquaculture Regulatory Science is currently collating information to address the second and third criteria (sockeye salmon susceptibility and temporal overlap) to determine the final list of pathogens to undergo a formal pathogen transfer risk assessment and to identify potential external experts to assist with the risk assessments of bacterial pathogens. The possibility of conducting risk assessments for multiple bacterial pathogens simultaneously is currently under consideration.

The next pathogen transfer risk assessments will be conducted under the same risk assessment framework and will follow the same conceptual model to ensure similarities between assessments and make a synthesis possible. In order to meet the government's commitment to respond to the Cohen Commission recommendations, this series of risk assessments, including the synthesis, need to be completed by the end of the 2019/2020 fiscal year.

Jay Parsons

Director, Aquaculture, Biotechnology and Aquatic Animal Health Science National Capital Region Page 1836
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page 1687

# Page 1837 is not relevant est non pertinente

Pathogen Transfer Risk Assessments in the Discovery Islands (bacteria causing systemic infections)

Components	Sten	Due By	Completed On	Bacteria	Kidney Disease (BKD)	Enteric Redmouth (ERM)	h (ERM)	Furunculosis	is	Salmonid rickttsial septicaemia (SRS)			
				Lead	Done (%)	Lead	Done (%)	Lead	Done (%)	Lead		2	
	Problem formulation (draft)	10-Nov-17	10-Nov-17	Caroline	100	Caroline	100	Caroline	1000	Caroline	1001		
	Meeting with client	10-Nov-17		Jay/Ingrid	09	Jay/Ingrid	09	Jay/Ingrid	09	Jay/Ingrid	99		
Problem	Engage with CFIA	10-Nov-17		Wayne/Jay	100	Wayne/Jay	too	Wayne/Jay	1001	Wavne/Jav	100		
formulation	Working group formation	17-Nov-17		Jay/Ingrid	75	Jay/Ingrid	75	Jay/Ingrid	7.5	Jay/Ingrid	75		
	Working group review of problem	30-Nov-17		Ingrid/Caroline	75	Ingrid/Caroline	75	Ingrid/Caroline	75	Ingrid/Caroline	7.5		
	Problem formulation (final)	8-Dec-17		Caroline	75	Caroline	75	Caroline	7.5	Caroline	7.5		
Communications	Update industry	TBD	ongoing	Jay/Ingrid	25	Jay/Ingrid	25	Jay/Ingrid	25	Jav/Ingrid	15		-
	Debrief NGOs, First Nations, province	TBD		Jay/Ingrid	0	Jay/Ingrid	10	Jav/Ingrid	01	Jav/Ingrid	100		
	Case definition	18-Oct-17	1-Nov-17	lan/Caroline/France	1008	lan/Caroline/France	(M)	lan/Caroline/France	100	an/Caroline/France	300		
n te	Contact industry	20-Oct-17	2-Nov-17	Jay	9	Jay	TOOL .	Jay	001	Jay	111000		
	Industry data acquisition	10-Nov-17		Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0		
	Industry data analysis	26-Jan-18		Caroline/France/epi?	0	Caroline/France/epi?	0	Caroline/France/epi?	0	Caroline/France/epi?	0		
	Outlines	2-Oct-17	2-Oct-17	Caroline		Caroline	1000	Caroline	DOT	Caroline	11.00		
	Identification of lead author	2-Oct-18	2-Oct-18	Ingrid/Caroline	100	Ingrid/Caroline	100	Ingrid/Caroline	100	ingrid/Caroline	11.400		
Pathogen working	Pathogen working paper (draft)	12-Jan-18		Linda Rhodes	75	Joy Wade	80	France Boily	35	Simon Jones	30		
nanor	Distribution for peer review	4-May-18		Caroline	0	Caroline	0	Caroline	0	Caroline	0		
	Pathogen working paper (reviewed)			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0		
	Pathogen working paper (approval)			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0		
	Pathogen working paper (final)	1-Sep-18		Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0		
	Risk assessment workshop	2-Feb-18		Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0	Ingrid/Caroline	0		
	Risk assessment (draft)	16-Mar-18		Caroline and WG	0	Caroline and WG	10	Caroline and WG	0	Caroline and WG	0		
-	Distribution for peer review	4-May-18		Caroline	0	Caroline	0	Caroline	0	Caroline	0		
working paper	Risk assessment (reviewed)			Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0		
	Risk assessment (approval)			Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0		
	Risk assessment (final)	1-Sep-18		Caroline and WG	0	Caroline and WG	0	Caroline and WG	0	Caroline and WG	0		
	Meeting agenda	4-May-18		Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0		
	Distribution of meeting material	4-May-18		Steering committee	0	Steering committee	0	Steering committee	0	Steering committee	0	BERTOLLINE THE STATE OF THE STA	
CSAS	Presentation for pathogen paper	15-Jun-18		Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0		
pvipw	Presentation for risk assessment	15-Jun-18		Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0		
	CSAS peer-review meeting	15-Jun-18		CSAS meeting chair	0	CSAS meeting chair	0	CSAS meeting chair	0	CSAS meeting chair	0		
	Reviewed pathogen paper			Linda Rhodes	0	Joy Wade	0	France Boily	0	Simon Jones	0		
	Reviewed risk assessment			Caroline	0	Caroline	0	Caroline	0	Caroline	0		
	Draft SAR			CSAS participants	0	CSAS participants	0	CSAS participants	0	CSAS participants	0		
	Revised SAR			Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0		
	Approval of pathogen paper			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0		
-1	Approval of risk assessment			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0		
	Approval of SAR			CSAS chair	0	CSAS chair	0	CSAS chair	0	CSAS chair	0		
Deliverables	Translation of SAR			CSAS office	0	CSAS office	0	CSAS office	0	CSAS office	0		
	Translation of figures included in SAR			Caroline/France	0	Caroline/France	0	Caroline/France	0	Caroline/France	0		
- 14	Submission of pathogen paper	1-Sep-18	1-Sep-18	Jay	0	Jay	0	Jay	0	Jay	0		
	Submission of risk assessment	1-Sep-18	1-Sep-18	Jay	0	Jay	0	Jay	0	Jay	0		
- 14	Submission of SAR (English)			Jay	0	Jay	0	Jay	0	Jay	0		
	Submission of SAR (Francais)			Jay	0	Jay	0	Jav	0	Jav	0		

# Fiche d'acheminement de correspondance Pêches et Océans Canada

CLASSIFICATION GCCMS #: 2017-007-00933 EKME #: 3787671

Wayne Moore

To:

Jean-François LaRue

Pour:

**Carmel Lowe** 

Andy Thompson

Date: September 22, 2018

Object: UPDATE ON PATHOGEN TRANSFER RISK ASSESSMENTS

Objet: IN THE DISCOVERY ISLANDS IN BRITISH COLUMBIA

From / Jay Parsons, Director, Aquaculture, Biotechnology and Aquatic Animal Health

De: Science Branch

Via:

Additional approvals:

Autre(s) approbation(s):

Material for the Senior Assistant Deputy Minister/Documents pour le Sous-ministre adjoint principal



Your Signature Votre signature



Information

Screen:

Filtre:

Remarks:

Remarques:

Distribution:

Rédacteur:

Caroline Mimeault (991-1439) / Ingrid Burgetz / Jay Parsons / db

#### Dickie, Catherine

From:

Miller-Saunders, Kristi

Sent:

December 18, 2017 10:24 AM

To:

Lowe, Carmel; Taylor, Nathan

Cc:

MacDougall, Lesley

Subject:

RE: question re RSR

Make sure the report does not say investigation, but rather inspection. I did not catch this previously.

Thanks

Kristi

From: Lowe, Carmel

Sent: December-18-17 10:04 AM

To: Miller-Saunders, Kristi; Taylor, Nathan

**Cc:** MacDougall, Lesley **Subject:** question re RSR

It is unclear in this sentence whether the virus is found in wild or farmed Coho, Chinook, Rainbow, Steelhead, Sockeye, Chum and Pink.... Can you clarify?

"Farmed and wild Atlantic Salmon (Salmo salar), Coho Salmon (Oncorhynchus kisutch), Chinook Salmon (Oncorhynchus tshawytscha), and Rainbow trout (Oncorhynchus mykiss), and wild Cutthroat Trout (Oncorhynchus clarkii), Steelhead Trout (Oncorhynchus mykiss), Sockeye Salmon (Oncorhynchus nerka), Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) have all tested positive for PRV through molecular testing."

#### Carmel

Carmel Lowe, Ph.D.
Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.qc.ca
Telephone | Téléphone 250-756-7177
Facsimile | Télécopieur 250-729-8360
Government of Canada | Gouvernement du Canada

#### Dickie, Catherine

From:

Lowe, Carmel

Sent:

December 18, 2017 10:28 AM

To:

Miller-Saunders, Kristi; Taylor, Nathan

Cc: Subject: MacDougall, Lesley RE: question re RSR

Ok perfect.

#### Carmel

Carmel Lowe, Ph.D.
Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360 Government of Canada | Gouvernement du Canada

From: Miller-Saunders, Kristi

Sent: Monday, December 18, 2017 10:23 AM

To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>; Taylor, Nathan < Nathan.Taylor@dfo-mpo.gc.ca>

Cc: MacDougall, Lesley < Lesley MacDougall@dfo-mpo.gc.ca>

Subject: RE: question re RSR

It is found in farmed and wild Atlantic, Coho, Chinook salmon and Rainbow trout, and the remaining species only tested in wild as they are not farmed

From: Lowe, Carmel

Sent: December-18-17 10:04 AM

To: Miller-Saunders, Kristi; Taylor, Nathan

Cc: MacDougall, Lesley
Subject: question re RSR

It is unclear in this sentence whether the virus is found in wild or farmed Coho, Chinook, Rainbow, Steelhead, Sockeye, Chum and Pink.... Can you clarify?

"Farmed and wild Atlantic Salmon (Salmo salar), Coho Salmon (Oncorhynchus kisutch), Chinook Salmon (Oncorhynchus tshawytscha), and Rainbow trout (Oncorhynchus mykiss), and wild Cutthroat Trout (Oncorhynchus clarkii), Steelhead Trout (Oncorhynchus mykiss), Sockeye Salmon (Oncorhynchus nerka), Chum Salmon (Oncorhynchus keta) and Pink Salmon (Oncorhynchus gorbuscha) have all tested positive for PRV through molecular testing."

Carmel

Carmel Lowe, Ph.D.
Regional Director Science | Directrice régionale des sciences
Fisheries and Oceans Canada | Pêches et Océans Canada
Pacific Biological Station | Station biologique du Pacifique
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel Lowe@dfo-mpo.gc.ca
Telephone | Téléphone 250-756-7177
Facsimile | Télécopieur 250-729-8360
Government of Canada | Gouvernement du Canada

# Ryan, Patricia

From:

Moore, Wayne

Sent:

December-18-17 3:18 PM

To:

Parsons, Jay

Subject:

Fw: Rapid Science Response.

Attachments:

RSR2017\_AQU01\_Browns\_and\_TofinoPRV\_Final\_for\_APPROVAL.DOCX; Appendix 1.docx;

Appendix 2A.xlsx; Appendix 2B.xlsx

**Categories:** 

**ATIP** 

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: Lowe, Carmel < Carmel.Lowe@dfo-mpo.qc.ca>

Sent: Monday, December 18, 2017 1:20 PM

To: McPherson, Arran

**Cc:** Moore, Wayne; MacDougall, Lesley **Subject:** Rapid Science Response.

Please find attached the rapid science response which I have just approved.

#### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360

Government of Canada | Gouvernement du Canada

# **Centre for Science Advice Pacific**

# FPP non-CSAS Request for Rapid Science Response

Request Contact:	Kristi Miller Saunders	Project Type: Aquaculture-Emergency response
Date of request:	November 29, 2017	Project footprint:
Region of proposed impact:	Tofino and Browns Bay, British Columbia	Habitat Type: Coastal
Purpose of request:	Information for Ministry of Environment / En	vironment and Climate Change Canada investigation
Potential affected species:	Pacific salmon	
Date required:	December 15, 2017	Request #:2017AQU01
	Required for ongoing inspection of fish processing for BC	acilities as conducted by Province

#### PROJECT OVERVIEW

In Canada, the regulation of processing of fish products is a shared Provincial – Federal responsibility. BC Ministry of Environment & Climate Change Strategies (ENV) supports marine fisheries and aquaculture and seafood industry development, issuing licenses to businesses involved in the aquaculture sector - including permits for seafood processing facilities - under the BC Fisheries Act and Fish Inspection Act. Thus, as a function of normally-operating fish processing plants, fish waste effluent is released back into the marine ecosystem subject to conditions of license.

Environment and Climate Change Canada (ECCC) administers section 36 of DFO's *Fisheries Act*, the key pollution prevention provision, prohibiting the deposit of deleterious substances into waters frequented by fish, unless authorized by regulations under the *Fisheries Act* or other federal legislation. A deleterious substance can be any substance that, if added to any water, would degrade or alter its quality such that it could be harmful to fish, fish habitat or the use of fish by people.

Following the public release of a video showing fish waste effluent being released into the marine environment, and subsequent related news articles and public interest, ENV initiated an inspection of two fish processing plants that were the subject of the videos. ENV has also initiated a compliance audit for fish processing plants.

The BC Ministry of Environment & Climate Change Strategies (ENV), Environmental Protection Regional Operations
Branch requested assistance from DFO to support the collection of water samples at two fish processing facilities and to
test collected effluent for the presence of the Piscine OrthoReovirus (PRV), as part of the ongoing inspection. Other labs
contacted at the time did not have the capacity to perform the necessary diagnostics on water samples.

# 1 QUESTION

#### Background:

Piscine OrthoReovirus (PRV) is known to be present in Norway, Japan, the United Kingdom, Ireland, Chile, the United States and Canada (Biering and Garseth 2012; Kibenge et al. 2013; Siah et al. 2015). Farmed and wild Atlantic Salmon (Salmo salar), Coho Salmon (Oncorhynchus kisutch), Chinook Salmon (Oncorhynchus tshawytscha), and Rainbow trout (Oncorhynchus mykiss), and wild Cutthroat Trout (Oncorhynchus clarkii), Steelhead Trout (Oncorhynchus mykiss),

# Pages 1845 to / à 1855 are withheld pursuant to section sont retenues en vertu de l'article

68(a)

of the Access to Information Act de la Loi sur l'accès à l'information

Parsons, Jay		
From:	Taylor, Nathan	
Sent:	Monday, December 18, 2017 6:10 PM	
То:	Parsons, Jay	
Cc:	McLeod, Patricia	
Subject:		
If you've got 30 mins to	call and discuss, we should.	
Trish – could   prevail o	on you to set something up please?	
Best		
NG		
From: Townsend, Jill Sent: Friday, December To: Taylor, Nathan Cc: Lowe, Carmel Subject:  Nathan,	r 15, 2017 5:19 PM	
401 Burrard Street   401 rue Cell   Gov **SOLICITOR CLIENT PRIV	igation Unit, Fisheries & Oceans Canada   Pêches et Océans Canada, Pacific Regional of Burrard, Pièce 200, Vancouver, BC V6C 3S4, Jill.Townsend@dfo-mpo.gc.ca Telephor ernment of Canada   Gouvernement du Canada / Gouvernem	ne   Téléphone (604) 658-2843, repared at the request of bying of the information is
saictly prombited.		s.16(2)(
		s.21(1)(
From: Bartlett, Michael		
Sent: December-15-17		s.23
To: Townsend, Jill; Ike		
Subject:		
Hi Jill:		

Michael K. Bartlett Legal Counsel **DFO-Legal Services** T:(613) 993-5791 B:(

From: Townsend, Jill

**Sent:** November-24-17 11:56 AM To: Ikejiani, Alexander; Bartlett, Michael

Subject:

Thanks Alex, and Michael.

Sent from my BlackBerry 10 smartphone on the Bell network.

From: Ikejiani, Alexander

Sent: Friday, November 24, 2017 8:42 AM To: Townsend, Jill; Bartlett, Michael

Subject:

Hello Jill,

### Regards

Alex Ikejiani Legal Counsel Department of Justice 200 Kent Street Ottawa ON K1A 0E6

Tel: (613) 991-6230

Email: alexander.ikejiani@dfo-mpo.gc.ca

Certified Specialist in Environmental Law

s.16(2)(c)

s.23

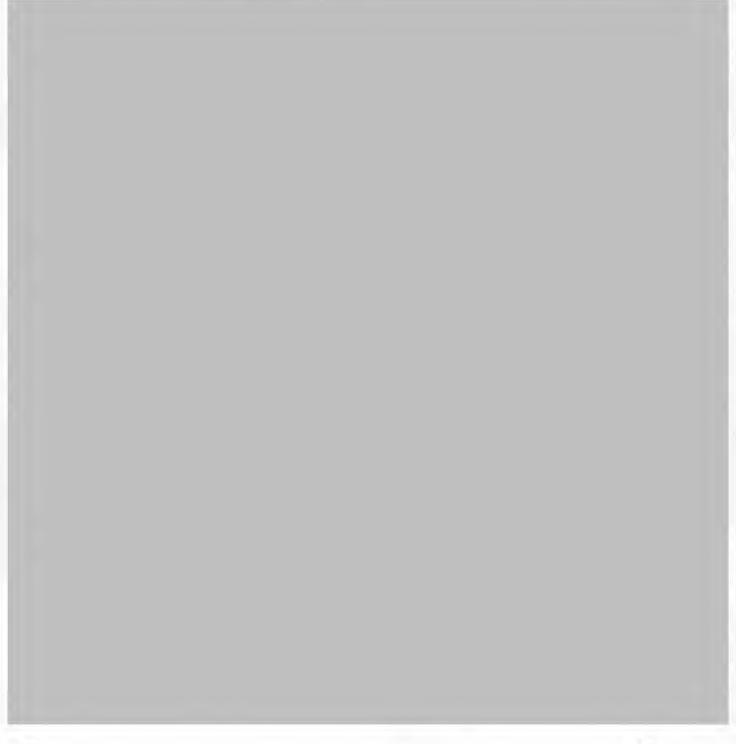
From: Townsend, Jill

**Sent:** November-23-17 10:32 PM

To: Ikejiani, Alexander

Subject:

Alex,	
ill Townsend	
itigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches et Océ 01 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, <u>Jill.Townse</u>	
Cell   Government of Canada   Gouvernement du Canada	
**SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNS counsel and the contents of this email are subject to solicitor client privilege	
strictly prohibited.**	
From: Townsend, Jill	
Sent: November-23-17 7:07 PM	
To: Taylor, Nathan Cc: Lowe, Carmel	
Subject:	
Hello Nathan,	
Teno (Vatriari,	
1914 T	
ill Townsend .itigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches et Océ	ans Canada, Pacific Regional Office   Région du Pacifique, 20
101 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, <u>Jill.Townse</u> Cell   Government of Canada   Gouvernement du Canada	nd@dfo-mpo.gc.ca Telephone   Téléphone (604) 658-2843,
**SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNS	EL): This email has been prepared at the request of
counsel and the contents of this email are subject to solicitor client privilege	. Any use, disclosure or copying of the information is
trictly prohibited.**	
From: Taylor, Nathan	
Sent: November-22-17 4:44 PM	s.16(2)(c)
To: Townsend, Jill Cc: Lowe, Carmel	s.21(1)(a)
Subject:	s.21(1)(b)
	s.23
li Jill,	



Best regards,

s.23

Nathan

Nathan G. Taylor, Ph.D.

Division Manager | Directeur de secteur

Aquatic Diagnostics Genomics and Technology Division | Division des diagnostics, la genomique, de la technologie aquatique

Fisheries and Oceans Canada | Peches et Oceans Canada

Pacific Biological Station | Station biologique du Pacifique 250-756-7395

### Miller-Saunders, Kristi

From: Miller-Saunders, Kristi

Sent: December-19-17 2:11 PM

To: Thomson, Andrew Subject: Talk I gave to Industry

Attachments: BCSFA\_Jaundice Talk KM-ED Nov 28, 2017\_Shortened-Comp.pptx

### Andrew,

Enclosed is the talk I gave at a BC Salmon Farmers Association meeting on PRV-HSMI state of knowledge workshop December 4th- 5th attended by Norwegian scientists, industry vets and leaders, BC and US Scientists, and DFO regulators. The talk outlines the most recent research out of my lab on PRV and linkages with disease in Pacific salmon. Key points are:

We have demonstrated that PRV infections in Chinook salmon can induce a host response that we have shown previously to be diagnostic of the presence of viral disease. This work was published in Conservation Physiology this year.

We demonstrate that 14% of moribund/dead farmed Chinook salmon on the west coast obtained though the DFO audit program were diagnosed with jaundice/anemia, a disease that around the world has been associated with various strains of PRV. There is only a single strain of PRV in BC, that which is known to cause HSMI in Atlantic salmon.

We show that throughout the developmental pathway of jaundice and across multiple affected tissues, PRV is localized within the regions and cells that become diseased, whether disease is through cell death (necrosis) in liver and kidney or inflammation in heart. We gave a similar talk on HSMI in Atlantic salmon and also demonstrated PRV localized with inflammatory lesions in heart and skeletal muscle tissue.

The primary infective tissue for PRV in both species is the red blood cells (which is why blood water from farmed fish is potentially a strong risk for PRV transmission to wild fish). We show that while PRV remains exclusively in the blood, even at high levels, it is tolerated and there is no disease response in the host. When the virus leaves the blood cells to infect other tissues/cells, it induces a disease response in the host.

The difference between HSMI in Atlantic salmon and jaundice/anemia in Chinook salmon is that in HSMI, PRV appears to leave the red blood cells without lysing (rupturing) them, whereas in Chinook salmon, there is massive lysis of red blood cells leading to anemia (pale gills and tissues) and overloading the kidney and liver with Heme from the breakdown of hemoglobin. Heme is processed in kidney and liver, but becomes toxic at high levels, leading to necrosis (death) of kidney tubules and hematocytes (liver cells), and a jaundice (yellowing) appearance in the fish. While we show that the virus also directly infects these cells, we suspect the heme overload, caused by PRV lysis of red blood cells, is likely the main mechanism leading to disease in jaundice fish. Liver and kidney are not highly affected in HSMI in Atlantic salmon, as the virus goes on to infect muscle cells (heart and skeletal) causing inflammation. This inflammatory response is present, but much reduced and very transient in Chinook salmon with jaundice. In fact the heart inflammation all bit disappears by the time the kidney becomes neurotic.

We have also demonstrated early (jaundice) disease development in wild Chinook salmon. There was a presentation by Dr. Maureen Purcell at the same meeting that showed an association of the same strain of PRV with a similar disease, which they and the Japanese call EIBS, in Washington State Coho salmon.

Kristi

Head, Molecular Genetics

Pacific Biological Station

Pages 1863 to / à 1894 are duplicates of sont des duplicatas des pages 1714 to / à 1745

### Miller-Saunders, Kristi

Vancouver, BC, Canada V6C 3S4

From: Sent: To:	Miller-Saunders, Kristi December-19-17 3:34 PM Thomson, Andrew
Subject:	RE: Rapid Science Response - aquaculture fish processing effluent testing
	from the upper levels highway. How late will be in the office? If I am late, can we meen need to get on the road right away?
From: Thomson, Andrew Sent: December 19, 2017 2:28 f To: Miller-Saunders, Kristi Subject: RE: Rapid Science Resp	PM  oonse - aquaculture fish processing effluent testing
That works fine.	
Andrew J L Thomson Regional Director   Directeur R	tégionale Fisheries Management Branch   Direction de la gestion des pêches
Original Message From: Miller-Saunders, Kristi Sent: Tuesday, December 19, 20 To: Thomson, Andrew <andrew rapid="" re:="" resp<="" science="" subject:="" td=""><td></td></andrew>	
Ok I would like to go over our latest	can walk down around 330 if that works. Of course timing depends on traffic. I findings with you.
Kristi	
From: Thomson, Andrew Sent: December 19, 2017 2:12 F To: Miller-Saunders, Kristi Subject: Re: Rapid Science Resp	PM  onse - aquaculture fish processing effluent testing
I can meet you this afternoon if	you want to come to the office.
Andrew J L Thomson	
	égionale Fisheries Management Branch   Direction de la gestion des pêches Pacific sheries & Oceans Canada   Pêches et Océans Canada
Suite 200 – 401 Burrard St.	

s.19(1)

andrew.thomson@dfo-mpo.gc.ca Telephone | Téléphone 604.666.0751 Facsimile | Télécopieur 250.666.8069 Government of Canada | Gouvernement du Canada.

Original Message

From: Miller-Saunders, Kristi

Sent: Tuesday, December 19, 2017 2:11 PM

To: Thomson, Andrew

Subject: RE: Rapid Science Response - aquaculture fish processing effluent testing

From: Thomson, Andrew

Sent: December 19, 2017 1:59 PM

To: Miller-Saunders, Kristi

Subject: Re: Rapid Science Response - aquaculture fish processing effluent testing

What time are you in Vancouver?

Andrew J L Thomson

Regional Director | Directeur Régionale Fisheries Management Branch | Direction de la gestion des pêches Pacific Region | Region du Pacifique Fisheries & Oceans Canada | Pêches et Océans Canada

Suite 200 – 401 Burrard St.

Vancouver, BC, Canada V6C 3S4
andrew.thomson@dfo-mpo.gc.ca
Telephone | Téléphone 604.666.0751
Facsimile | Télécopieur 250.666.8069
Government of Canada | Gouvernement du Canada.

Original Message

From: Miller-Saunders, Kristi

Sent: Tuesday, December 19, 2017 1:54 PM

To: Thomson, Andrew Cc: Lowe, Carmel

Subject: RE: Rapid Science Response - aquaculture fish processing effluent testing

#### Hello Andrew,

I sent Amy Tabata to do the sampling with the inspectors from the other agencies and they noted that there was no chemical treatment in either facility and from what Amy was told it was not a requirement of their license. Interestingly I was at an industry meeting when the bloodwater story broke and said that marine harvest does chlorinate their effluent but others do not necessarily. If there was enough chlorination to kill the virus it would likely also degrade the DNA but given the amount of organic material in their crudely filtered bloodwater it would be doubtful that chlorination alone could kill virus. You are correct in questioning whether or not PCR alone could ascertain whether the virus is still viable. I told them that this could be questioned if samples were chemically treated hence we froze several samples for future I infectivity testing just in case. However given lack of treatment and the known robustness of this virus I don't see any reason to suggest the virus would not be viable.

will be in Vancouver this afternoon if you want to get together.

#### Kristi

From: Thomson, Andrew

Sent: December 19, 2017 1:22 PM

To: Miller-Saunders, Kristi

Cc: Lowe, Carmel

Subject: FW: Rapid Science Response - aquaculture fish processing effluent testing

Kristi

- 1) I'm around this week if you want to pick a time to talk.
- 2) On this rapid science advice, it doesn't appear there was any form of secondary effluent treatment or chlorine treatment at these facilities. However I'm curious that if there was a secondary treatment or some form of chlorine treatment that deactivated the virus, would the PCR test still show positive?

#### Andrew J L Thomson

Regional Director | Directeur Régionale Fisheries Management Branch | Direction de la gestion des pêches

From: Trudeau, Miriam

Sent: Tuesday, December 19, 2017 12:49 PM

To: Thomson, Andrew <Andrew.Thomson@dfo-mpo.gc.ca>; Antcliffe, Bonnie <Bonnie.Antcliffe@dfo-mpo.gc.ca>

Cc: Kaba, Kyle <Kyle.Kaba@dfo-mpo.gc.ca>

Subject: FW: Rapid Science Response - aquaculture fish processing effluent testing

Not sure if you received these.

From: McPherson, Arran

Sent: Tuesday, December 19, 2017 12:28 PM

To: Trudeau, Miriam < Miriam.Trudeau@dfo-mpo.gc.ca < mailto: Miriam.Trudeau@dfo-mpo.gc.ca >>

Cc: Hopkins, Lillian <Lillian.Hopkins@dfo-mpo.gc.ca<mailto:Lillian.Hopkins@dfo-mpo.gc.ca>>; Jenkins, Phil <Phil.Jenkins@dfo-mpo.gc.ca<mailto:Phil.Jenkins@dfo-mpo.gc.ca>>; White, Andrea <Andrea.White@dfo-

mpo.gc.ca<mailto:Andrea.White@dfo-mpo.gc.ca>>

Subject: Rapid Science Response - aquaculture fish processing effluent testing

Miriam, I believe MINO was advised that DFO Science was doing some testing of aquaculture fish processing plant effluent at the request of the province of BC.

To close the loop, the results are enclosed and have been (or very shortly will be) provided to the province. In general, the results are that PRV was detected in the samples. However, given the prevalence of PRV in farmed populations and presence of the virus in blood, it was expected that blood effluent from processing plants would contain PRV.

Arran.

Ryan, Patricia	
From:	Moore, Wayne
Sent:	December-19-17 6:02 PM
To:	Parsons, Jay
Subject:	Fw: HEAD's UP: Interview request (Pacific region) - The Tyee - aquaculture/PRV
Importance:	High
Categories:	ATIP
ţ	
Sent from my BlackBer	ry 10 smartphone on the Rogers network.
Sent: Tuesday, December To: LaRue, Jean-François Cc: Jenkins, Phil; Nielsen	; Moore, Wayne; McPherson, Arran; Morel, Philippe
Pls see below.	
Sent: Tuesday, December To: Gareau, Laura; Lavig Cc: MacNeil, Vince; Amla	ne, Kevin ni, Ashraf; Hopkins, Lillian; Malko, Carol; Quinn, Caroline; Trudeau, Miriam; Hubley, Marian;
NCR Media RCN (DFO/MF	Phil; Smith, Kathleen; Fagan, Ashley; Bate, Dan; Rainer, Michelle; Chow, Vance; Gilbert, Sarah; PO) terview request (Pacific region) - The Tyee - aquaculture/PRV
•	
HEAD's up Laura :	
Issue: Reporter Operations Vet, DFO Aquinatcheries are point sour	The Tyee has written to Dr. Ian Keith, Field laculture Management Division with questions based on his opinion that processing plants and rees for PRV.
	has received a copy of the reporter's questions, for information. The reporter is hoping to hear ith for a science based interview.
Questions:	

- 1. In November, 2016, you wrote to a colleague: "Processing plants and hatcheries are point sources for PRV. If there is to [be] sustainable aquaculture the processing plants must treat effluent, and keep any infectious disease out, respectively...If enhancement hatcheries are exempt, this is 19th century thinking." Has any action been taken by DFO or any other entity to your knowledge since that time to address these concerns? Do you remain concerned about processing plants and hatcheries as point sources for PRV?
- 2. What is the nature of these concerns for you? In other words, what is the consequence of additional sources for PRV? Are you concerned about the potential effect of the virus on wild salmon? Please feel free to address both

hatcheries and processing plants separately (i.e. what are your specific concerns with respect to the transfer of hatchery smolts with PRV into salmon farm pens and what are your concerns with respect to PRV-contaminated blood water being discharged into the marine environment)

- 3. Recent news reports indicated Minister LeBlanc was launching a review in the wake of media attention on blood water discharge from two farmed fish processing plants. Are you involved in this investigation and can you provide me with any details on the investigation?
- 4. Can you explain your relationship with BC's Animal Health Centre? I understand they audit fish health at salmon farms under some form of agreement with DFO. Do you, as a DFO veterinarian, provide any oversight to their work? How might you typically engage with them as they conduct their work with regards to farmed fish?
- 5. The recent (2017) Wessel paper in PLOS One established an etiological link between PRV and HSMI do you accept that PRV causes HSMI and to what degree or under what circumstances would you accept that this is the case?

Ryan, Patricia	
From:	Moore, Wayne
Sent:	December-20-17 10:22 AM
To:	Parsons, Jay
Cc:	Taylor, Nathan
Subject:	
•	privileged solicitor client
Attachments:	
Importance:	High
For info and sharing with	regional colleagues who may not have it.
From: McPherson, Arran	
Sent: December 20, 2017	
	ne.Moore@dfo-mpo.gc.ca>
Subject: FW:	privileged solicitor client
Importance: High	
From: Trudeau, Miriam	
Sent: Wednesday, Decem	nher 20, 2017 9:49 AM
	rran.McPherson@dfo-mpo.gc.ca>; Sharzer, Stephen < <u>Stephen.Sharzer@dfo-mpo.gc.ca</u> >;
	e.Antcliffe@dfo-mpo.gc.ca>; Thomson, Andrew < <u>Andrew.Thomson@dfo-mpo.gc.ca</u> >; Morel,
Philippe < Philippe. Morel	
	n.Hopkins@dfo-mpo.gc.ca>; Butcher, Ashley <a href="mailto:Ashley.Butcher@dfo-mpo.gc.ca">Ashley.Butcher@dfo-mpo.gc.ca</a> ; Malko, Carol
	gc.ca>; Jarjour, Jasmine < <u>Jasmine.Jarjour@dfo-mpo.gc.ca</u> >
Subject: FW:	privileged solicitor client
Importance: High	
Miriam	
From: Valerio, Michael	
Sent: Wednesday, Decen	nber 20, 2017 7:31 AM
**	iriam.Trudeau@dfo-mpo.gc.ca>
	ie.Cocking@dfo-mpo.gc.ca>; XNCR-Grp, CA / AC < <u>XNCR-GrpCA/AC@dfo-mpo.gc.ca</u> >; Boudreau
	Boudreau-Brown@dfo-mpo.gc.ca>; Ménard, Angèle < Angele.Menard@dfo-mpo.gc.ca>;
	e.Fadden@dfo-mpo.gc.ca>; Hubley , Marian < Marian.Hubley@dfo-mpo.gc.ca>
Subject: Fw:	- privileged solicitor client
Jubject. FW.	priving Sea solicitor circuit
Hi Miriam,	
I'm flagging this corres	pondence sent by Pacific last night. MCU
will input into system.	
	s.19(1)
	s.21(1)(a)
	1

s.21(1)(b)

s.23

The technical briefing today may have some helpful lines  Comms is attending the technical briefing? I can follow up were	with them.
Thanks,	
Sent from my BlackBerry 10 smartphone on the Rogers net  From: pac.prmc / pac.urpcm (DFO/MPO) < XPAC.PRMCU@dfo-m Sent: Tuesday, December 19, 2017 9:15 PM To: Minister / Ministre (DFO/MPO) Cc: Valerio, Michael Subject: FW:  The attached was forwarded to me	a dangan mengupum nyuggan mengupunggan pangan pang
From: Townsend, Jill Sent: Tuesday, December 19, 2017 5:38 PM To: pac.prmc / pac.urpcm (DFO/MPO) Subject: Hi,	- privileged solicitor client
Have you seen the attached yet? Or, started a draft reply for DFO if we can expect a draft soon I am not trying to I'm sure it will make its way to you,	O to the attached yet? I am sending this fyi – and to see enter this into the system – just an info enquiry here.
Jill Townsend Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches e 401 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, Jill.To Cell   Government of Canada   Gouvernement du Canada **SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF CC counsel and the contents of this email are subject to solicitor client privistrictly prohibited. **	wnsend@dfo-mpo.gc.ca Telephone   Téléphone (604) 658-2843,  DUNSEL): This email has been prepared at the request of
From: pac.prmc / pac.urpcm (DFO/MPO) Sent: December-19-17 5:15 PM To: Townsend, Jill Subject:	
Thanks. I'll add that.	
From: Townsend, Jill Sent: Tuesday, December 19, 2017 4:43 PM To: pac.prmc / pac.urpcm (DFO/MPO) Cc: Ikejiani, Alexander Subject: Importance: High	s.16(2)(c)
Hello Candace,	s.21(1)(a) s.21(1)(b)

I was concerned that our draft reply had no response — so I mysel in yellow highlighting, perhaps prefaced by something like, "In respon	f prefer including the additional reply noted above use to the concerns you've raised
Jill Townsend Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches et Océa 401 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, Jill.Townser Cell   , Government of Canada   Gouvernement du Canada **SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNSE counsel and the contents of this email are subject to solicitor client privilege. strictly prohibited.**	nd@dfo-mpo.gc.ca Telephone   Téléphone (604) 658-2843,  EL): This email has been prepared at the request of
From: pac.prmc / pac.urpcm (DFO/MPO) Sent: December-19-17 3:18 PM To: Townsend, Jill Subject:	
Hi Jill	
to see it?	Is there a particular person who needs
Thanks	
Candace.	
From: Townsend, Jill	e entre entr
Sent: Tuesday, December 19, 2017 2:38 PM To: pac.prmc / pac.urpcm (DFO/MPO)	
Cc: Russow, Theona Subject:	
Importance: High	
Hello, I am following up on the below.	
Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada   Pêches et Océa 401 Burrard Street   401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, Jill.Townset Cell   Government of Canada   Gouvernement du Canada **SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNSI counsel and the contents of this email are subject to solicitor client privilege. strictly prohibited.**	nd@dfo-mpo.gc.ca Telephone   Téléphone (604) 658-2843, EL): This email has been prepared at the request of
	s.21(1)(a) s.21(1)(b)
	s.21(1)(b)

From: Russow, Theona

Sent: December-18-17 3:25 PM

To: Townsend, Jill

Subject:

Importance: High

And this one.

Thanks, Theona

Theona Russow | Litigation Manager | Fisheries & Oceans Canada | Pêches et Océans Canada | Pacific Regional Office | Région du Pacifique | 200- 401 Burrard Street | 401 rue Burrard, Pièce 200 | Vancouver, BC V6C 3S4 | Theona.Russow@dfo-mpo.gc.ca | Telephone | Téléphone (604) 666-0776 | Cell | Government of Canada | Gouvernement du Canada

\*\*SOLICITOR CLIENT PRIVILEGE NOTICE (EMAIL AT THE REQUEST OF COUNSEL): This email has been prepared at the request of counsel and the contents of this email are subject to solicitor client privilege. Any use, disclosure or copying of the information is strictly prohibited.\*\*

From: pac.prmc / pac.urpcm (DFO/MPO)

Sent: December-18-17 3:23 PM

To: Russow, Theona Cc: Keefe, Marisa Subject: \*\*

Importance: High

#### Hi Theona:

(NOTE: I have sent it to AQ to review at the same time; I don't anticipate changes there as it's mostly approved text.)

Please use track changes to insert any necessary edits in the attached document, and FORWARD the document to XPAC PRMCU.

Your review should also include technical accuracy and consistency with policy direction.

Thanks,

Candace

\*\*Please direct all e-mails regarding Minister's (001) and RDG's (501) correspondence to XPAC PRMCU.\*\*

Candace McGuire, Heather Fowlie & Allison Murray

Managers, Pacific Region Ministerial Correspondence Unit Fisheries and Oceans Canada/Government of Canada XPACPRMCU@dfo-mpo.gc.ca Tel 604-666-0823

s.16(2)(c) s.21(1)(a)

Gestionnaires de la correspondance ministériel Pêches et Océans Canada/Gouvernement du Canada

s.21(1)(b)

XPACPRMCU@dfo-mpo.gc.ca Tel 604-666-0823

s.23

# Pages 1904 to / à 1911 are withheld pursuant to sections sont retenues en vertu des articles

21(1)(b), 23, 21(1)(a)

of the Access to Information Act de la Loi sur l'accès à l'information

### Mimeault, Caroline

From:

Mimeault, Caroline

Sent:

December 21, 2017 1:00 PM

To:

Parsons, Jay; Garver, Kyle

Subject:

Draft answer to media request

Attachments:

MECTS-#3863619-v1-2017

\_SRS\_ABAAHS\_-\_Answers\_to\_media\_request\_(The\_National\_Observer).DOCX

Importance:

High

First drat. Please have a look.

**Kyle** – especially the 2<sup>nd</sup> and 4th question. Answers from the 5<sup>th</sup> question came from previous briefings.

### **Answers for Media Request**

Reporter, The National Observer

December 20th, 2017

-- "the likelihood for juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms is estimated to be extremely unlikely with reasonable uncertainty given current health management practices (i.e., vaccination, surveillance for early detection and depopulation) that limit the amount of potential IHNV shed into the environment from infected farms."

DFO is reasonably uncertain that it is extremely unlikely? Is there a way someone could reword this so I understand what that means? Much appreciated.

There were several steps in the likelihood assessment. For each step, the likelihood and the uncertainty were reported separately. The uncertainty is due to both the natural variability of biological systems as well as knowledge gaps in the scientific literature. Where there were knowledge gaps, expert opinion and models were used, and the uncertainty reported reflects how much each step was based on scientific information, models or expert opinion.

Based on available data, it was concluded that it is extremely unlikely for wild fish to get infected by IHNV released from the farms as the estimated maximum concentrations would be 7,000 to 100,000 times lower than the concentrations required for infecting juvenile sockeye salmon.

However, given some knowledge gaps related to the IHNV concentrations on farms during an outbreak which was estimated through modeling and knowledge gaps related to the time juvenile sockeye salmon spend in net pens and the outcome of a prolonged exposure of juvenile Sockeye Salmon to sublethal doses which was addressed through expert opinion, the conclusion for this specific step were determined to be reasonably uncertain.

However, and most importantly, despite high uncertainties in some area of the risk assessment, there is minimal risk to Fraser River sockeye salmon mainly because it is very unlikely that an IHN outbreak on Atlantic salmon farms in the Discovery Islands would occur given that there has been no IHN outbreak on Atlantic salmon farms in the Discovery Islands since 2003 and given the current effective fish health management practices in place which includes vaccination of all farmed Atlantic salmon against IHNV.

-- Will DFO produce similar reports for IHNV and the likelihood of infection for other wild Pacific salmon populations? Why or why not?

At this point in time, there are no plans to produce a similar report on IHNV in other Pacific salmon. The next pathogen transfer risk assessments will continue to focus on the risk to Fraser River sockeye salmon resulting from pathogens that caused diseases on Atlantic salmon in the Discovery Islands.

However, although the risk assessment focused on the risk to Fraser River sockeye salmon, considerations were given to other fish species susceptible to IHN. Given that other Pacific salmon species are either less susceptible to IHN than sockeye salmon or even not susceptible to this disease, it had no impact on the outcome of the risk assessment.

-- In its section on assumptions, DFO says it assumes all regulations, prevention measures, and vaccination rules are being followed by Atlantic salmon farmers when it comes to IHNV. Did it take any steps to verify this?

Regulations and licence conditions include the requirement for a Salmonid Health Management Plan and accompanying proprietary Standard Operating Procedures which are reviewed and approved by DFO as a part of the initial licence application. Additionally, DFO conducts audits of farms under the Fish Health Audit and Surveillance Program on a regular basis. During the audits, the activities, protocols and procedures are reviewed and fish sample are collected for histopathology, bacteriology, virology and molecular diagnostics. A summary of the audit results are available on the DFO website.

It is in the interest of the company to ensure the health of farmed fish. To that end, in addition to conditions of licence, Atlantic salmon companies operating in the Discovery Islands developed and agreed to a Salmon Farming Industry Viral Disease Management Plan which includes the vaccination of all farmed Atlantic salmon against IHN and procedures to respond to an incidence. additionally, all active Atlantic salmon farms in the Discovery Islands are certified by third parties that require that all fish be vaccinated against diseases for which effective vaccines are available.

-- When was the last time IHNV was detected in Pacific salmon, which kind of salmon, and in what kind of numbers? (ie: how bad was the problem?)

IHNV is endemic to the Pacific Northwest, meaning it is native to the environment.

Long-term monitoring of British Columbia sockeye salmon stocks from the Skeena, Fraser, and Columbia River watersheds conducted by DFO revealed that annual prevalence of IHNV in spawning adult sockeye salmon highly variable within and among stocks. For example, between 1987 and 2015, prevalence of IHNV in spawning adults varied from 0 to 50% (average of 9%) in Weaver Creek and from 0 to 62% (average of 11%) in Nadina River.

Prevalence of IHNV in juvenile Fraser River sockeye salmon collected by DFO during surveys conducted in the Strait of Georgia and Discovery Islands in May, June and July of 2010 to 2015 varied from 0 to 10.5% during their out-migration through the Strait of Georgia and Discovery Islands.

All Pacific salmon tested for IHNV by the Canadian Food Inspection Agency between 2012 and 2014 as part of a program to survey wild and enhanced anadromous salmonids in British Columbia were negative for IHNV.

While it occurs in wild salmon, farmed Atlantic salmon are more susceptible to the disease. However, there have been no IHN outbreaks on Atlantic salmon farms in the Discovery Islands since 2003 or in BC since 2012. There has been no detection of IHNV in any farmed Atlantic salmon vaccinated against IHN.

# -- Can DFO provide a brief update on efforts to combat PRV and HSMI in wild salmon, transferred from farmed salmon?

DFO will be conducting additional risk assessments in the Discovery Islands including an assessment of heart lesions reported on Atlantic salmon farms which will include considerations of PRV and HSMI.

Currently, there are a number of important research projects underway on PRV, its relationship to disease, and its effects on fish. These studies are being led by DFO researchers on both sides of the country.

Laboratory studies in Canada and the US aimed at investigating the disease causing potential of PRV have resulted in different outcomes than those demonstrated in Norway. Differences between Norwegian and North American studies may be due to PRV strain differences, environmental factors, and/or other variables influencing stress and host disease resistance. This is an ongoing, active area of research by DFO.

One DFO study has found evidence of HSMI lesions on a BC Atlantic salmon farm, however, there was no associated elevation in fish mortality. The disease was associated with the presence of PRV, and while this does not mean that PRV causes HSMI, the co-presence is an important finding. The issue of causality, while very recently confirmed in Norway, is still an area of scientific investigation in Canada given the different virus strains, different fish stocks and the lack of success in forcing a disease response.

To date PRV appears to have high transmissibility but low virulence in wild Pacific salmon. However, DFO continues to investigate the disease causing potential of PRV to better understand what effects (if any) PRV has on wild Pacific salmon. Our research is also looking at the presence of other diseases such as jaundice and how they might be related to PRV. The experience of other countries suggests that there is often a complex combination of virus, environmental conditions and host (fish) diseases responses required to result in fish mortality.

Canadians can be confident that their Government is taking this question seriously and we will continue to report our findings and scientific conclusions publically and in a timely manner.

### Mimeault, Caroline

From:

Mimeault. Caroline

Sent:

December 21, 2017 1:23 PM

To:

Parsons, Jay; Garver, Kyle

Subject:

RE: Draft answer to media request

**Attachments:** 

MECTS-#3863619-v2-2017

\_SRS\_ABAAHS\_-\_Answers\_to\_media\_request\_(The\_National\_Observer).DOCX

Importance:

High

Hi,

I made some changes – attached.

To facilitate review, I highlighted the sections for which I would appreciate your feedback Kyle.

I am also copying them below to make it easier:

# -- Will DFO produce similar reports for IHNV and the likelihood of infection for other wild Pacific salmon populations? Why or why not?

Although the risk assessment focused on the risk to Fraser River sockeye salmon, considerations were given to other fish species susceptible to IHN. Pacific salmon species were not of concern given that they are either less susceptible to IHN than sockeye salmon or not even susceptible to this disease.

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While it occurs in wild salmon, farmed Atlantic salmon are more susceptible to the disease. However, there have been no IHN outbreaks on Atlantic salmon farms in the Discovery Islands since 2003 or in BC since 2012. There has been no detection of IHNV in any farmed Atlantic salmon vaccinated against IHN.

From: Mimeault, Caroline

**Sent:** December 21, 2017 1:00 PM **To:** Parsons, Jay; Garver, Kyle

Subject: Draft answer to media request

Importance: High

First drat. Please have a look.

**Kyle** – especially the 2<sup>nd</sup> and 4th question. Answers from the 5<sup>th</sup> question came from previous briefings.

### **Answers for Media Request**

Reporter, The National Observer

December 20th, 2017

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At this point in time, there are no plans to produce a similar report on IHNV in other Pacific salmon. The next pathogen transfer risk assessments will continue to focus on the risk to Fraser River sockeye salmon resulting from pathogens that caused diseases on Atlantic salmon in the Discovery Islands.

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Canadians can be confident that their Government is taking this question seriously and we will continue to report our findings and scientific conclusions publically and in a timely manner.

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-21-17 3:02 PM

To:

Parsons, Jay; Mimeault, Caroline

Subject:

RE: media request on salmon report

Not to put too find a point on it, I think the PRV one is fine. With regards to #1, is the short answer?

All other things being equal, based on the evidence available, we believe it is extremely unlikely that ....

Total uncertainty includes both variability, which is a function of the system that is not reducible with additional measurements, and lack of knowledge that can be reduced with additional data or expert opinion (Vose, 2008). Reasonable uncertainty means ...

On #2, why do we not mention Cohen in the response?

On #3, I think we should lead with "Yes, we do check"

From: Parsons, Jay

Sent: December 21, 2017 2:13 PM

**To:** Moore, Wayne < Wayne. Moore@dfo-mpo.gc.ca> **Subject:** FW: media request on salmon report

Importance: High

Wayne, FYI. This will be coming your way soon. Deadline 3 pm. Responses that Caroline and I have developed for some follow up questions from yesterday's briefing. Jay

From: Mimeault, Caroline

Sent: Thursday, December 21, 2017 2:12 PM

To: Jenkins, Phil; Parsons, Jay

Cc: Saindon, Carole

Subject: RE: media request on salmon report

Importance: High

Attached.

From: Jenkins, Phil

**Sent:** December 21, 2017 1:34 PM **To:** Parsons, Jay; Mimeault, Caroline

Cc: Saindon, Carole

Subject: RE: media request on salmon report

Many thanks Jay...I will run it past Wayne and Arran, likely just as an fyi. Then media relations will run up the rest of the way...and then to the journalist.

Phil

From: Parsons, Jay

Sent: December-21-17 1:26 PM

To: Jenkins, Phil < Phil.Jenkins@dfo-mpo.gc.ca>; Mimeault, Caroline < Caroline.Mimeault@dfo-mpo.gc.ca>

Cc: Saindon, Carole < Carole.Saindon@dfo-mpo.gc.ca>

Subject: RE: media request on salmon report

We are working on it. Waiting for Kyle's input. What approval steps do we need to go through. Should I run this by Wayne and Arran or will you?

Jay

From: Jenkins, Phil

Sent: Thursday, December 21, 2017 12:53 PM

To: Parsons, Jay: Mimeault, Caroline

Cc: Saindon, Carole

Subject: RE: media request on salmon report

Hi all...just checking in...how's progress?

From: Jenkins, Phil

Sent: December-21-17 9:18 AM

To: Parsons, Jay < <u>Jay.Parsons@dfo-mpo.gc.ca</u>>

Cc: Garver, Kyle < Kyle.Garver@dfo-mpo.gc.ca >; Mimeault, Caroline < Caroline.Mimeault@dfo-mpo.gc.ca >; Burgetz,

Ingrid < <a href="mailto:Ingrid.Burgetz@dfo-mpo.gc.ca">Ingrid <a href="mailto:Ingrid <a href="mailto:Ingrid.Burgetz@dfo-mpo.gc.ca">Ingrid <a href="mailto:Ingrid <a href="mailto:Ingrid <a href="mailto:Ingrid.Burgetz@dfo-mpo.gc.ca">Ingrid <a href="mailto:Ingrid <a href="mailto:Ingr

Subject: FW: media request on salmon report

Good morning Jay,

We have a follow up media call, below. Deadline is 3 pm today.

I would have cut and pasted our media lines in...but I'm not sure as written and approved, they answer her particular questions.

Over to you for help on this.

Phil

From: Saindon, Carole

Sent: December-21-17 8:58 AM

To: Gilbert, Sarah <Sarah.Gilbert@dfo-mpo.gc.ca>; Chow, Vance <Vance.Chow@dfo-mpo.gc.ca>; Jenkins, Phil

<Phil.Jenkins@dfo-mpo.gc.ca>

Cc: Sankey, Lauren < Lauren. Sankey@dfo-mpo.gc.ca >; Burelle, Marie-Pier < Marie-Pier. Burelle@dfo-mpo.gc.ca >; NCR

Media RCN (DFO/MPO) < Media. XNCR@dfo-mpo.gc.ca>

Subject: Re: media request on salmon report

Thx! Copying Phil for follow up with Jay's group.

Sent from my BlackBerry 10 smartphone on the Rogers network.

From: NCR Media RCN (DFO/MPO) Sent: jeudi 21 décembre 2017 8:52 AM To: Saindon Carole: Gilhert Sarah: Chow Vance

Cc: Sankey, Lauren; Burelle, Marie-Pier

Subject: FW: media request on salmon report

Wasn't sure if this one had already been dealt with...(?)

V.

From:

**Sent:** December-20-17 4:50 PM **To:** NCR Media RCN (DFO/MPO)

Subject: re: media request on salmon report

Good afternoon,

here from the National Observer. I hope you're all well.

I'm writing with a deadline of 3 p.m. Eastern on Thursday regarding the IHNV report on Fraser River sockeye published today.

I'm not sure I understand the following sentence:

-- "the likelihood for juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms is estimated to be **extremely unlikely** with **reasonable uncertainty** given current health management practices (i.e., vaccination, surveillance for early detection and depopulation) that limit the amount of potential IHNV shed into the environment from infected farms."

DFO is reasonably uncertain that it is extremely unlikely? Is there a way someone could reword this so I understand what that means? Much appreciated.

- -- Will DFO produce similar reports for IHNV and the likelihood of infection for other wild Pacific salmon populations? Why or why not?
- -- In its section on assumptions, DFO says it assumes all regulations, prevention measures, and vaccination rules are being followed by Atlantic salmon farmers when it comes to IHNV. Did it take any steps to verify this?
- -- When was the last time IHNV was detected in Pacific salmon, which kind of salmon, and in what kind of numbers? (ie: how bad was the problem?)
- -- Can DFO provide a brief update on efforts to combat PRV and HSMI in wild salmon, transferred from farmed salmon?

Thanks for your help!

Best,

s.19(1)

Reporter, <u>The National Observer</u> Ottawa | Vancouver | Toronto

Twitter | Website | Blog

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-21-17 3:43 PM

To:

Jenkins, Phil

Cc:

Parsons, Jay; White, Andrea

Subject:

FW: FYI: Follow up media call re: CSAS RA MECTS-#3863619-v3-2017

Attachments:

SRS ABAAHS - Answers to media request\_(The\_Nation....docx

Importance:

High

I am both today....I have added the underlined stuff if people are ok with this.

### **Answers for Media Request**

Reporter, The National Observer

December 20th, 2017

-- "the likelihood for juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms is estimated to be extremely unlikely with reasonable uncertainty given current health management practices (i.e., vaccination, surveillance for early detection and depopulation) that limit the amount of potential IHNV shed into the environment from infected farms."

DFO is reasonably uncertain that it is extremely unlikely? Is there a way someone could reword this so I understand what that means? Much appreciated.

### To clarify...

Based on the available evidence and current management practices, we are confident that it is extremely unlikely that juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms.

Our uncertainty assessment is a best science practice which comments on the quality of the scientific evidence and the variability of the natural system.

#### To elaborate:

There were four steps in the likelihood assessment. Each step was first assessed individually and conclusions of all steps were then combined for an overall assessment. The overall conclusion for the likelihood assessment was that it is extremely unlikely that juvenile Fraser River Sockeye Salmon will be infected and become diseased due to IHNV released from Atlantic salmon farms.

In addition, the level of uncertainty was assessed for each step. Uncertainty reflects how much each step was based on scientific information, models or expert opinion. There was reasonable uncertainty related to the overall likelihood assessment given some knowledge gaps related to the IHNV concentrations on farms during an outbreak which was estimated through modeling and knowledge gaps related to the time juvenile sockeye salmon spend around farms.

However, and most importantly, despite uncertainties in some area of the risk assessment, there is minimal risk to Fraser River sockeye salmon mainly because it is very unlikely that an IHN outbreak on Atlantic salmon farms in the Discovery Islands would occur given that there has been no IHN outbreak on Atlantic salmon farms in the Discovery

Islands since 2003 and given the current effective fish health management practices in place which includes vaccination of all farmed Atlantic salmon against IHNV.

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At this point in time, there are no plans to produce a similar report on IHNV in other Pacific salmon. The next pathogen transfer risk assessments will continue to focus on the risk to Fraser River sockeye salmon resulting from pathogens that caused diseases on Atlantic salmon in the Discovery Islands as recommended by the Cohen Commission.

Although the risk assessment focused on the risk to Fraser River sockeye salmon, considerations were given to other fish species susceptible to IHN. Pacific salmon species were not of concern given that they are either less susceptible to IHN than sockeye salmon or not even susceptible to this disease.

-- In its section on assumptions, DFO says it assumes all regulations, prevention measures, and vaccination rules are being followed by Atlantic salmon farmers when it comes to IHNV. Did it take any steps to verify this?

Yes we regularly check a number of these items. Under the Pacific Aquaculture Regulations, the licence conditions include the requirement for a Salmonid Health Management Plan and accompanying Standard Operating Procedures which are reviewed and approved by DFO as a part of the initial licence application. Additionally, DFO conducts audits of farms under the Fish Health Audit and Surveillance Program on a regular basis. During the audits, the activities, protocols and procedures are reviewed and fish samples are collected for pathogen diagnosis. A summary of the audit results are available on the DFO website.

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From: Jenkins, Phil

Sent: December 21, 2017 3:03 PM

**To:** McPherson, Arran <a href="mailto:Arran.McPherson@dfo-mpo.gc.ca">Arran.McPherson@dfo-mpo.gc.ca</a>; Moore, Wayne <Wayne.Moore@dfo-mpo.gc.ca> **Cc:** Saindon, Carole <Carole.Saindon@dfo-mpo.gc.ca>; White, Andrea <Andrea.White@dfo-mpo.gc.ca>

Subject: FYI: Follow up media call re: CSAS RA

Importance: High

Hi Arran and Wayne,

We had a follow up call on the briefing yesterday. Jay's group has put together this response attached. Please flag any show-stoppers...

Thanks,

Phil

Phil Jenkins

A/Mgr. | Gestionnaire intérimaire

Strategic Communications – Ecosystems and Oceans Science | Communications stratégiques – Sciences des écosystèmes et des océans

Fisheries and Oceans Canada | Pêches et Océans Canada Communications (Science)

613-991-0323

s.19(1)

s.21(1)(b)

### **Answers for Media Request**

Reporter, The National Observer

December 20th, 2017

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In addition, the level of uncertainty was assessed for each step. Uncertainty reflects how much each step was based on scientific information, models or expert opinion. There was reasonable uncertainty related to the overall likelihood assessment given some knowledge gaps related to the IHNV concentrations on farms during an outbreak which was estimated through modeling and knowledge gaps related to the time juvenile sockeye salmon spend around farms.

However, and most importantly, despite uncertainties in some area of the risk assessment, there is minimal risk to Fraser River sockeye salmon mainly because it is very unlikely that an IHN outbreak on Atlantic salmon farms in the Discovery Islands would occur given that there has been no IHN outbreak on Atlantic salmon farms in the Discovery Islands since 2003 and given the current effective fish health management practices in place which includes vaccination of all farmed Atlantic salmon against IHNV.

-- Will DFO produce similar reports for IHNV and the likelihood of infection for other wild Pacific salmon populations? Why or why not?

At this point in time, there are no plans to produce a similar report on IHNV in other Pacific salmon. The next pathogen transfer risk assessments will continue to focus on the risk to Fraser River sockeye salmon resulting from pathogens that caused diseases on Atlantic salmon in the Discovery Islands.

Although the risk assessment focused on the risk to Fraser River sockeye salmon, considerations were given to other fish species susceptible to IHN. Pacific salmon species were not of concern given that they are either less susceptible to IHN than sockeye salmon or not even susceptible to this disease.

-- In its section on assumptions, DFO says it assumes all regulations, prevention measures, and vaccination rules are being followed by Atlantic salmon farmers when it comes to IHNV. Did it take any steps to verify this?

Under the Pacific Aquaculture Regulations, the licence conditions include the requirement for a Salmonid Health Management Plan and accompanying Standard Operating Procedures which are reviewed and approved by DFO as a part of the initial licence application. Additionally, DFO conducts audits of farms under the Fish Health Audit and Surveillance Program on a regular basis. During the audits, the activities, protocols and procedures are reviewed and fish samples are collected for pathogen diagnosis. A summary of the audit results are available on the DFO website.

It is in the interest of the company to ensure the health of farmed fish. To that end, in addition to meeting conditions of licence, Atlantic salmon companies operating in the Discovery Islands developed and agreed to a Salmon Farming Industry Viral Disease Management Plan which includes procedures to prevent or minimize the spread of disease in the event of an outbreak. This agreement includes the vaccination of all farmed Atlantic salmon against IHN. Additionally, all active Atlantic salmon farms in the Discovery Islands are certified by a third party eco-certification program that requires all fish to be vaccinated against diseases for which effective vaccines are available.

-- When was the last time IHNV was detected in Pacific salmon, which kind of salmon, and in what kind of numbers? (ie: how bad was the problem?)

IHNV is endemic to the Pacific Northwest, meaning it is native to the environment.

Long-term monitoring of British Columbia sockeye salmon stocks from the Skeena, Fraser, and Columbia River watersheds conducted by DFO revealed that annual prevalence of IHNV in spawning adult sockeye salmon is highly variable within and among stocks. For example, between 1987 and 2015, prevalence of IHNV in spawning adults varied from 0 to 50% (average of 9%) in Weaver Creek and from 0 to 62% (average of 11%) in Nadina River.

Prevalence of IHNV in juvenile Fraser River sockeye salmon collected by DFO during surveys conducted in the Strait of Georgia and Discovery Islands in May, June and July of 2010 to 2015 varied from 0 to 10.5% during their out-migration through the Strait of Georgia and Discovery Islands.

All Pacific salmon tested for IHNV by the Canadian Food Inspection Agency between 2012 and 2014 as part of a program to survey wild and enhanced anadromous salmonids in British Columbia were negative for IHNV.

While it occurs in wild salmon, farmed Atlantic salmon are more susceptible to the disease. However, there have been no IHN outbreaks on Atlantic salmon farms in the Discovery Islands since 2003 or in BC since 2012. There has been no detection of IHNV in any farmed Atlantic salmon vaccinated against IHN.

-- Can DFO provide a brief update on efforts to combat PRV and HSMI in wild salmon, transferred from farmed salmon?

DFO will be conducting additional risk assessments in the Discovery Islands including an assessment of the risks to Fraser River sockeye salmon associated with heart lesions reported on Atlantic salmon farms. This assessment will include considerations of PRV and HSMI.

Currently, there are a number of important research projects underway on PRV, its relationship to disease, and its effects on fish. These studies are being led by DFO researchers on both sides of the country.

Laboratory studies in Canada and the US aimed at investigating the disease causing potential of PRV have resulted in different outcomes than those demonstrated in Norway. Differences between Norwegian and North American studies may be due to PRV strain differences, environmental factors, and/or other variables influencing stress and host disease resistance. To date PRV appears to have high transmissibility but low virulence in wild Pacific salmon. This is an ongoing, active area of research by DFO.

### Ryan, Patricia

From:

Moore, Wayne

Sent:

December-21-17 3:57 PM

To:

Mimeault, Caroline: Jenkins, Phil

Cc:

Parsons, Jay

Subject:

RE: FYI: Follow up media call re: CSAS RA

I am fine with this version fill with one change. I think we need to be clear that the answer to #3 is Yes we do check and then go over details.

From: Mimeault, Caroline

Sent: December 21, 2017 3:55 PM

To: Jenkins, Phil < Phil.Jenkins@dfo-mpo.gc.ca>; Moore, Wayne < Wayne.Moore@dfo-mpo.gc.ca>

**Cc:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca> **Subject:** RE: FYI: Follow up media call re: CSAS RA

Attached including Wayne and Kyle's comments highlighted in yellow.

From: Parsons, Jay

Sent: December 21, 2017 3:46 PM

To: Mimeault, Caroline

Subject: Fw: FYI: Follow up media call re: CSAS RA

Importance: High

From: Moore, Wayne

Sent: Thursday, December 21, 2017 03:42 PM

To: Jenkins, Phil

Cc: Parsons, Jay; White, Andrea

Subject: FW: FYI: Follow up media call re: CSAS RA

I am both today....I have added the underlined stuff if people are ok with this.

#### **Answers for Media Request**

Reporter, The National Observer

December 20th, 2017

-- "the likelihood for juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms is estimated to be extremely unlikely with reasonable uncertainty given current health management practices (i.e., vaccination, surveillance for early detection and depopulation) that limit the amount of potential IHNV shed into the environment from infected farms."

DFO is reasonably uncertain that it is extremely unlikely? Is there a way someone could reword this so I understand what that means? Much appreciated.

To clarify...

Based on the available evidence and current management practices, we are confident that it is extremely unlikely that juvenile Fraser River Sockeye Salmon to be infected and become diseased due to IHNV released from Atlantic Salmon farms.

Our uncertainty assessment is a best science practice which comments on the quality of the scientific evidence and the variability of the natural system.

#### To elaborate:

There were four steps in the likelihood assessment. Each step was first assessed individually and conclusions of all steps were then combined for an overall assessment. The overall conclusion for the likelihood assessment was that it is extremely unlikely that juvenile Fraser River Sockeye Salmon will be infected and become diseased due to IHNV released from Atlantic salmon farms.

In addition, the level of uncertainty was assessed for each step. Uncertainty reflects how much each step was based on scientific information, models or expert opinion. There was reasonable uncertainty related to the overall likelihood assessment given some knowledge gaps related to the IHNV concentrations on farms during an outbreak which was estimated through modeling and knowledge gaps related to the time juvenile sockeye salmon spend around farms.

However, and most importantly, despite uncertainties in some area of the risk assessment, there is minimal risk to Fraser River sockeye salmon mainly because it is very unlikely that an IHN outbreak on Atlantic salmon farms in the Discovery Islands would occur given that there has been no IHN outbreak on Atlantic salmon farms in the Discovery Islands since 2003 and given the current effective fish health management practices in place which includes vaccination of all farmed Atlantic salmon against IHNV.

-- Will DFO produce similar reports for IHNV and the likelihood of infection for other wild Pacific salmon populations? Why or why not?

At this point in time, there are no plans to produce a similar report on IHNV in other Pacific salmon. The next pathogen transfer risk assessments will continue to focus on the risk to Fraser River sockeye salmon resulting from pathogens that caused diseases on Atlantic salmon in the Discovery Islands <u>as recommended by the Cohen Commission</u>.

Although the risk assessment focused on the risk to Fraser River sockeye salmon, considerations were given to other fish species susceptible to IHN. Pacific salmon species were not of concern given that they are either less susceptible to IHN than sockeye salmon or not even susceptible to this disease.

-- In its section on assumptions, DFO says it assumes all regulations, prevention measures, and vaccination rules are being followed by Atlantic salmon farmers when it comes to IHNV. Did it take any steps to verify this?

Yes we regularly check a number of these items. Under the Pacific Aquaculture Regulations, the licence conditions include the requirement for a Salmonid Health Management Plan and accompanying Standard Operating Procedures which are reviewed and approved by DFO as a part of the initial licence application. Additionally, DFO conducts audits of farms under the Fish Health Audit and Surveillance Program on a regular basis. During the audits, the activities, protocols and procedures are reviewed and fish samples are collected for pathogen diagnosis. A summary of the audit results are available on the DFO website.

It is in the interest of the company to ensure the health of farmed fish. To that end, in addition to meeting conditions of licence, Atlantic salmon companies operating in the Discovery Islands developed and agreed to a Salmon Farming Industry Viral Disease Management Plan which includes procedures to prevent or minimize the spread of disease in the event of an outbreak. This agreement includes the vaccination of all farmed Atlantic salmon against IHN. Additionally, all active Atlantic salmon farms in the Discovery Islands are certified by a third party eco-certification program that requires all fish to be vaccinated against diseases for which effective vaccines are available.

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DFO will be conducting additional risk assessments in the Discovery Islands including an assessment of the risks to Fraser River sockeye salmon associated with heart lesions reported on Atlantic salmon farms. This assessment will include considerations of PRV and HSMI.

Currently, there are a number of important research projects underway on PRV, its relationship to disease, and its effects on fish. These studies are being led by DFO researchers on both sides of the country.

Laboratory studies in Canada and the US aimed at investigating the disease causing potential of PRV have resulted in different outcomes than those demonstrated in Norway. Differences between Norwegian and North American studies may be due to PRV strain differences, environmental factors, and/or other variables influencing stress and host disease resistance. To date PRV appears to have high transmissibility but low virulence in wild Pacific salmon. This is an ongoing, active area of research by DFO.

From: Jenkins, Phil

Sent: December 21, 2017 3:03 PM

To: McPherson, Arran < Arran.McPherson@dfo-mpo.gc.ca >; Moore, Wayne < Wayne.Moore@dfo-mpo.gc.ca >

Cc: Saindon, Carole < Carole.Saindon@dfo-mpo.gc.ca >; White, Andrea < Andrea.White@dfo-mpo.gc.ca >

Subject: FYI: Follow up media call re: CSAS RA

Importance: High

Hi Arran and Wayne,

We had a follow up call on the briefing yesterday. Jay's group has put together this response attached. Please flag any show-stoppers...

Thanks,

Phil

s.19(1)

s.21(1)(b)

Phil Jenkins

A/Mgr. | Gestionnaire intérimaire

Strategic Communications – Ecosystems and Oceans Science | Communications stratégiques – Sciences des écosystèmes et des océans

Fisheries and Oceans Canada | Pêches et Océans Canada Communications (Science)

613-991-0323

Ryan, Patricia		
From: Sent: To: Cc: Subject:	Moore, Wayne December-27-17 2:00 PM Lowe, Carmel Parsons, Jay	
Let us know if we can be of assi	stance.	
Cc: Thomson, Andrew < Andrew	IcPherson@dfo-mpo.gc.ca>; Reid, I	Rebecca <rebecca.reid@dfo-mpo.gc.ca> e, Wayne <wayne.moore@dfo-mpo.gc.ca>; Taylor, sley.MacDougall@dfo-mpo.gc.ca&gt;</wayne.moore@dfo-mpo.gc.ca></rebecca.reid@dfo-mpo.gc.ca>
Arran & Rebecca,		
See below		
I will provide an estimate of exp	pected completion	once all staff are back on strength from the hols.
Carmel  Carmel Lowe, Ph.D.  Regional Director Science   Director Scien	Pêches et Océans Canada on biologique du Pacifique	s.21(1)(a) s.21(1)(b) s.23

Carmel.Lowe@dfo-mpo.gc.ca
Telephone | Téléphone 250-756-7177
Facsimile | Télécopieur 250-729-8360
Government of Canada | Gouvernement du Canada

From: Townsend, Jill

Carmel, and Andy,

Sent: Friday, December 22, 2017 3:53 PM

To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>; Thomson, Andrew < Andrew.Thomson@dfo-mpo.gc.ca>
Cc: Lavigne, Lauren < Lauren.Lavigne@dfo-mpo.gc.ca>; Taylor, Nathan < Nathan.Taylor@dfo-mpo.gc.ca>; Owens, Heather < Heather.Owens@dfo-mpo.gc.ca>; Jackson, Corey < Corey.Jackson@dfo-mpo.gc.ca>; Ikejiani, Alexander < Alexander Ikejiani@dfo-mpo.gc.ca>

Heather < Heather. Owens@dfo-mpo.gc.ca >; Jackson, Corey < Corey. Jackson@dfo-mpo.gc.ca >; Ikejiani, Alexander < Alexander. Ikejiani@dfo-mpo.gc.ca > Subject:

Importance: High

#### Jill Townsend

Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada | Pêches et Océans Canada, Pacific Regional Office | Région du Pacifique, 200-401 Burrard Street | 401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, <a href="mailto:lill.Townsend@dfo-mpo.gc.ca">Jill.Townsend@dfo-mpo.gc.ca</a> Telephone | Téléphone (604) 658-2843, Cell | Government of Canada | Gouvernement du Canada

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s.16(2)(c)

s.21(1)(a)

s.21(1)(b)

s.23

## Pages 1937 to / à 1940 are withheld pursuant to section sont retenues en vertu de l'article

23

Ryan, Patricia		
From: Sent: To:	Moore, Wayne December-27-17 2:01 PM Parsons, Jay	
Subject:		
Attachments:		
Importance:	High	
fyi		
Cc: Thomson, Andrew <a< td=""><td>rran.McPherson@dfo-mpo.gc.ca&gt;; Reid, Reb</td><td>Vayne &lt; Wayne. Moore@dfo-mpo.gc.ca&gt;; Taylor,</td></a<>	rran.McPherson@dfo-mpo.gc.ca>; Reid, Reb	Vayne < Wayne. Moore@dfo-mpo.gc.ca>; Taylor,
Arran & Rebecca,		
I will provide an estimate	of expected completion	once all staff are back on strength from the hols.
Carmel		s.21(1)(a) s.21(1)(b)
Carmel Lowe, Ph.D. Regional Director Science	e   Directrice régionale des sciences	s.23

Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

Carmel.Lowe@dfo-mpo.gc.ca

Telephone | Téléphone 250-756-7177 Facsimile | Télécopieur 250-729-8360 Government of Canada | Gouvernement du Canada

From: Townsend, Jill

Sent: Friday, December 22, 2017 3:53 PM

To: Lowe, Carmel < Carmel.Lowe@dfo-mpo.gc.ca>; Thomson, Andrew < Andrew.Thomson@dfo-mpo.gc.ca>
Cc: Lavigne, Lauren < Lauren.Lavigne@dfo-mpo.gc.ca>; Taylor, Nathan < Nathan.Taylor@dfo-mpo.gc.ca>; Owens, Heather < Heather.Owens@dfo-mpo.gc.ca>; Jackson, Corey < Corey.Jackson@dfo-mpo.gc.ca>; Ikejiani, Alexander < Alexander.Ikejiani@dfo-mpo.gc.ca>

Subject:

Importance: High

Carmel, and Andy,

Jill	Townsend	

Litigation Case Manager, Litigation Unit, Fisheries & Oceans Canada | Pêches et Océans Canada, Pacific Regional Office | Région du Pacifique, 200-401 Burrard Street | 401 rue Burrard, Pièce 200, Vancouver, BC V6C 3S4, <a href="mailto:lill.Townsend@dfo-mpo.gc.ca">lill.Townsend@dfo-mpo.gc.ca</a> Telephone | Téléphone (604) 658-2843, Cell | Government of Canada | Gouvernement du Canada

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s.16(2)(c)

s.21(1)(a)

5.21(1)(a)

s.21(1)(b)

s.23

## Pages 1943 to / à 1946 are withheld pursuant to section sont retenues en vertu de l'article

23

Pages 1947 to / à 1954 are duplicates of sont des duplicatas des pages 1904 to / à 1911

# Pages 1955 to / à 1959 are withheld pursuant to sections sont retenues en vertu des articles

21(1)(b), 23, 21(1)(a)

### Page 1960

is withheld pursuant to sections est retenue en vertu des articles

21(1)(b), 16(2)(c), 23, 21(1)(a)

## Page 1961 is withheld pursuant to section est retenue en vertu de l'article

23

### McLeod, Patricia

Sec.	 		
ş.,	O	m	• •

Lowe, Carmel

Sent:

December 28, 2017 2:44 PM

To:

McPherson, Arran

Cc:

Thomson, Andrew; Taylor, Nathan; Moore, Wayne

Subject:

RE: draft email

Attachments:

This time with attachments...

#### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

s.14(a) s.19(1)

s.21(1)(a)

s.21(1)(b)

Carmel.Lowe@dfo-mpo.gc.ca

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Government of Canada | Gouvernement du Canada

s.23

From: Lowe, Carmel

Sent: Thursday, December 28, 2017 2:41 PM

To: McPherson, Arran < Arran. McPherson@dfo-mpo.gc.ca >

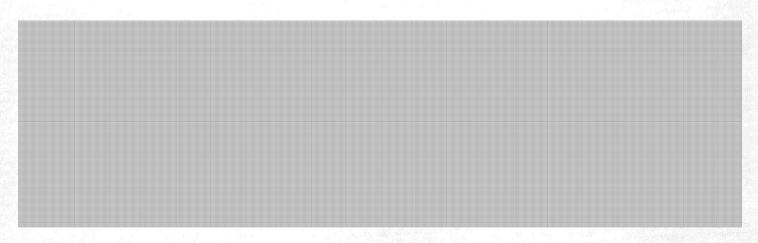
Cc: Thomson, Andrew < Andrew. Thomson@dfo-mpo.gc.ca >; Taylor, Nathan < Nathan. Taylor@dfo-mpo.gc.ca >; Moore,

Wayne < Wayne. Moore@dfo-mpo.gc.ca>

Subject: FW: draft email

Arran,

Want to provide you with an update



#### Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada Pacific Biological Station | Station biologique du Pacifique 3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

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Government of Canada | Gouvernement du Canada

s.14(a)

s.19(1)

s.21(1)(a)

s.21(1)(b)

s.23

## Pages 1964 to / à 1970 are withheld pursuant to section sont retenues en vertu de l'article

23